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# Antibacterial and Anticancer activity of Glycolipid Biosurfactant from Manglicolous Yeast *Geotrichum candidum* PV 37

K. A. Nimsi and K. Manjusha*

*Department of Marine Biosciences, Faculty of Ocean Science and Technology, Kerala University of Fisheries and Ocean Studies Panangad, Madavana, Kochi - 682 506, India.*
**Corresponding Author Email: manjusha.k@kufos.ac.in, manjushak.kufos@gmail.com*

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## ABSTRACT

**Bioprospecting potentials of manglicolous yeasts are noteworthy. They have been considered a potent source of biosurfactants. In this perspective, 99 strains (PV1-99) isolated from the mangrove forest of Puthuvype, Kerala, India were screened for the production of biosurfactants. These yeasts were analyzed by the oil displacement test with 5 different oils (sunflower, olive, gingelly, diesel, coconut). Out of these 99 isolates, PV 37 *Geotrichum candidum* was found to be the most potent strain. The physical and chemical characterization of the biosurfactant of PV 37 revealed that it was glycolipid in nature. This was confirmed by Fourier transform infrared spectroscopy (FTIR) analysis. The maximum yield of the biosurfactant obtained was 2.8 g/L after 120 hrs of incubation. The biosurfactant exhibited stability in a wide range of temperatures, pH, and salinity. In the prevailing scenario of multidrug resistance and limitations of chemotherapeutic agents, the search is on for safe therapeutic agents. As the biosurfactant of PV 37 displayed cytotoxicity against breast cancer cell line MCF-7 as well as antibacterial against MDR Gram-negative bacteria, it indicates its possible application in the pharmaceutical industry**

**Keywords:** Agriculture, Biosurfactant, Yeast, Antibacterial, Anticancer, *Geotrichum candidum*, Glycolipid

## INTRODUCTION

Surfactants are amphipathic compounds having hydrophobic and hydrophilic moieties (Banat *et al.*, 2010), and can be used in a variety of industries like food, petroleum, agriculture, and, pharmaceuticals, etc (Thakur *et al.*, 2021). An increase in synthetic surfactant usage has negatively affected human health and the environment. Consequently, microbial surface-active compounds (MSAC) were introduced as potential alternatives for sustainable development (Pontevedra-Pombal *et al.*, 2013). They are referred to as biosurfactants (BS). The BS classes include glycolipids, lipopeptides, and lipoproteins (Desai and Banat, 1997). Amongst all, the low molecular weight BS are the most studied (Paulino *et al.*, 2016). Glycolipid consists of a long fatty acid chain with carbohydrate compounds (Müller and Hausmann, 2011). Microbial glycolipids have been found to possess multifunctional properties (Inès and Dhouha, 2015). Glycolipids are potent in destabilizing the biological membranes and activating/inhibiting enzymes, which leads to antimicrobial and antioxidant activity (Thakur *et al.*, 2021).

Scientific reports point out that bacteria are the main producers of BS (Martins and Martins, 2018). However, the pathogenic nature of most of these organisms has prompted a search for alternative promising microbial candidates (Jimoh and Lin, 2019). Despite making up only 19% of all BS, fungal biosurfactants are discovered to be more chemically and structurally adaptable (Nimsi *et al.*, 2022). As they come from generally recognized as safe (GRAS) strains and have benefits including low environmental toxicity, eco-friendly, biodegradability, and resistance to severe environments, yeast biosurfactants have recently attracted commercial interest. Yeast BS has been reported mainly from *Yarrowia* sp., *Pseudozyma* sp., and *Candida* sp. (Nimsi *et al.*, 2022). Cancer and the spread of multidrug resistance (MDR) are the two major clinical concerns these days. Cancer remains the leading cause of death worldwide, which contributed to 10 million deaths in 2022 (WHO 2020). Chemotherapy is an anticancer treatment with some limitations like chemoresistance and side effects (Sak, 2012; Dy and Adjei, 2013). MDR has emerged as a public health threat all over the world (Tanwar *et al.*, 2014). Chifiriuc *et al*. 2022 in their study noted the resemblances between antimicrobial and chemoresistance mechanisms and also that they share common targets and mechanisms of action.

In this prevailing scenario, the search is on for safe therapeutic agents. Therefore in, this study,we concentrated on the extraction and characterization of BS from *Geotrichum candidum* PV 37, isolated from Puthuvype mangroves and assessed the antibacterial and anticancer properties.

## MATERIALS AND METHODS

### Strains Used for the Study

Ninety-nine yeasts strains (PV1-PV99) were isolated as part of the KARP-funded project "A study on the diversity of manglicolous yeasts of KUFOS campuses" from 13 different mangrove tree species found in the Puthuvype (PV) mangroves located in central Kerala, India were used for the study.

### Screening of Biosurfactant Production

The strains were screened for biosurfactant (BS) production in YM media (glucose 1g, malt extract 0.3 g, peptone 0.5 g, yeast extract 0.3 g, seawater 100 ml). The ability of purified colonies to produce BS was determined by the oil displacement test with 5 different oils (sunflower, olive, gingelly, diesel, coconut). Thirty milliliters of distilled water and 50 µl of oil were taken in a Petri plate. Cell-free Supernatant (50 µl) was added to the center of the oil. The diameter of the zone of displacement on the addition of supernatant was measured as a measure of biosurfactant production (Patel and Patel, 2020).

**Production of biosurfactant**

The isolate PV 37 which was found to be the most potent among 99 isolates was further studied for biosurfactant production. PV 37 was initially pre-cultured in 10 ml of YM (media and incubated for 7 days in a rotary shaker for 120 rpm at $30^0$ C or 7 days. This served as a pre-inoculum. From this 10ml was transferred to the 100 ml of modified YM broth (glucose replaced with sunflower oil) and incubated for 7 days in a rotary shaker for 120 rpm at $30^0$ C or 7 days.

**Physical and chemical characteristics of BS**

**Physical characterization**

***Parafilm test***

Fifty microliters of the culture supernatant from the production medium were placed on the strip of Parafilm M and the diameter was noted. The shape of the supernatant drop on the surface of parafilm M was examined after 1min. If the drop becomes flat, it indicates the presence of biosurfactant in the supernatant. If the drop retains the dome shape, it indicates a negative result (Patel and Patel, 2020).

***Emulsification index measurement***

To determine the Emulsification index, 4 ml diesel was added to 4 ml of culture supernatant, vortexed for 2 minutes, and allowed to stand for 24 hrs. The emulsification index was calculated using the formula,

E 24= Height of emulsification layer / Total height x100

**Chemical characterization**

***Phenol sulphuric acid test***

One ml of 5% phenol was added to 1 ml of cell-free culture supernatant. To this mixture, 4 drops of concentrated $H_2SO_4$ were added. The development of orange color with the addition of reagent indicates the presence of glycolipids. (Kalyani and Sireesha, 2014)

***Biuret test***

Two ml of cell-free culture supernatant was heated at 70º C for 10 minutes and 10 drops of 1 M NaOH were added to this. To this mixture, 1% Copper sulfate solution was added drop by drop. The appearance of a violet or pink ring indicates the presence of lipopeptides

***Phosphate test***

To 2 ml of culture supernatant, 10 drops of 6 M Nitric acid were added and heated at 70º C for 10 minutes. Yellow color development after the addition of 5% ammonium molybdate solution is indicative of the presence of phospholipids.

**Extraction and Purification of Biosurfactant**

BS production was carried out in shaker flasks. After incubation the culture broth was centrifuged at 10,000 rpm for 10 min and the supernatant was collected in a beaker. One molar of $H_2SO_4$ was used to adjust the pH of the supernatant to 2 (Muthezhilan *et al.*, 2014). To this supernatant chloroform: methanol (2:1) was added and kept in a beaker overnight covered with aluminum foil with holes in it. Then, the crude BS was separated by high-speed centrifugation of the acidified supernatant (12 000×*g*; 4º C; 20 min). On evaporation of the solvent, the biosurfactant precipitated as a white-colored powder (Sekar *et al.*, 2010)**.**

**Growth kinetics**

To obtain insight into yeast growth and BS production, the strain was grown on a sunflower oil medium. The samples were retrieved to measure optical density, the diameter of the displacement zone (OST), and E24 and BS yield.

**Stability Study of BS**

The effect of various environmental factors such as temperature, salinity, and pH on the stability of the BS was evaluated. The stability of the BS was determined by incubating the BS solution (0.1mg/ml) at a wide range of temperatures (4–100º C), pH (4.0–10.0), and NaCl concentration (0 – 25.0 %, w/v) for 30 min and then determining the OST.

**Characterization of extracted BS**

The extracted BS was then investigated for its chemical structure by thin-layer chromatography (TLC), and Fourier transform infrared spectroscopy (FTIR).

**TLC**

BS was separated on a silica gel plate using chloroform: methanol: glacial acetic acid (65:15:4, v/v/v) as the mobile phase. The iodine vapors were used to detect the lipid fraction of BS. Plates were heated at 110º C for 10 min after the application of the spraying agents. The iodine vapours and Molish reagent were used to detect BS fractions. Plates were heated at 110º C for 10 min after application of the spraying agents.

**FTIR**

FTIR is one of the best methods to determine the types of functional groups and chemical bonds in the structure of an unknown compound. The FTIR spectrum of the BS was recorded by FTIR spectrophotometer (PerkinElmer Frontier; USA) at 400–4000 $cm^{-1}$.

**Antimicrobial activity**

**Disc diffusion method**

The extracted biosurfactant was dissolved in sterile distilled water (1mg/ml) and the antimicrobial activity was checked against bacterial pathogens (Microbial Type Culture Collection (MTCC), Chandigarh, India) *Escherichia coli, Pseudomonas* sp., *Bacillus* sp., *Vibrio* sp., *Aeromonas* sp., *Salmonella* sp., *Klebsiella* sp., *and Staphylococcus* sp. by disc diffusion method. Sterile discs (Himedia) were impregnated with 20µl biosurfactant. The impregnated discs were placed on test cultures swabbed onto Mueller Hinton Agar and incubated for 24 hrs at $37^0$ C. SDS and water were taken as positive and negative control respectively.

**Cell lysis method**

Test bacterial cultures(5ml) OD was adjusted to 1, to this 1ml of BS (1mg/ml) was added and incubated for 30 minutes. Test bacterial cultures incubated with Sodium Dodecyl Sulphate, served as positive control whereas sterile water was the negative control. After incubation, the OD was measured spectroscopically at 600 nm.

**Antioxidant activity**

For the antioxidant assays, the BS was dissolved in water, to prepare various concentrations (20, 40, 60, 80, and 100 µg/ml) and assayed for antioxidant activity. All experiments were conducted in triplicates. The antioxidant activity of PV 37 biosurfactant was determined using Diphenyl-2-picrylhydrazyl (DPPH) scavenging activity (DPPH) assay and 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) radical assay (Mukherjee *et al.*, 2017).

**Evaluation of cytotoxicity**

**Cytotoxicity Assessment with Cancer cell lines**

The cell line/cell lines were procured from the National Centre for Cell Sciences (NCCS), Pune, India. The cells were cultured in Dulbecco's Modified Eagles Medium (DMEM-Himedia), supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS) and 1% antibiotic cocktail containing Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). The cell-containing flasks ($25cm^2$) were incubated at $37^OC$ at a 5% $CO_2$ environment with humidity in a cell culture incubator (Galaxy® 170 Eppendorf, Germany). The cells (2500 cells/well) were seeded on 96 well plates and allowed to acclimatize to the culture conditions such as 37 °C and 5% CO2 environment in the incubator for 24 h. The test samples were prepared in DMEM media (10 mg/mL) and filter sterilized using a 0.2 µm Millipore syringe filter. The samples were further diluted in DMEM media and added to the wells containing cultured cells at final concentrations of 6.25, 12.5, 25, 50, and 100µg/mL respectively. Untreated wells were kept in control. All the experiments were done in triplicate and average values were taken to minimize errors. After treatment with the test samples the plates were further incubated for 24 h. After the incubation period, the media from the wells were aspirated and discarded. 100 µL of 0.5 mg/mL MTT solution in PBS was added to the wells. The plates were further incubated for 2h for the development of formazan crystals. The supernatant was removed and 100 µL DMSO (100%) was added per well. The absorbance at 570 nm was measured with a micro plate reader. Two wells per plate without cells served as blank. All the experiments were done in triplicates. The cell viability was

expressed using the following formula:

% of cell viability = (Average absorbance of treated/ Average absorbance of control) X 100

**Molecular Identification**

The genomic DNA of the potent BS-producing yeast strain PV37 was isolated and purified (Kutty and Phillip, 2008). The ITS region was amplified using the primers ITS-1 and ITS-4 (Forward ITS 1-5' TCC GTA GGT GAA CCT GCG G 3' and Reverse ITS 4-5' TCCTCC GCT TAT TGA TAT GC 3') (White *et al.*, 1990). The sequences obtained above were aligned by using BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST). The sequences which shared over 99% similarity with currently available sequences are considered to be the same species and phylogenetic trees were constructed by MEGA11.0.

**RESULTS AND DISCUSSION**

Among the 99 yeast strains 65 showed oil displacement activity and the isolate PV 37 exhibited the maximum zone of displacement **(Table 1)** and this strain was used for further study. The oil displacement test is an indirect measure of the surface activity of a surfactant sample tested against an oil; a larger diameter of the zone of displacement indicates a higher surface activity of the testing solution (Rodrigues *et al.*, 2006).

In the parafilm M test, the culture supernatant drop retained its shape and did not flatten (6mm) and it exhibited an emulsification index of 49% in the E24 test. This confirmed the presence of extracellular bio-emulsifier in the cell-free culture supernatant.

**Table 1**: Screening for the BS production with different oils

| Isolates | Diesel | Olive oil | Sunflower | Mustard | Gingelly | Isolates | Diesel | Olive oil | Sunflower | Mustard | Gingelly |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **PV1** | 6mm | - | 2mm | 3mm | 9mm | **PV28** | 15mm | 10mm | 3mm | - | - |
| **PV2** | - | 3mm | 5mm | 9mm | 15mm | **PV29** | - | 15mm | 4mm | 12mm | - |
| **PV3** | - | - | 9mm | 5mm | 12mm | **PV30** | 5mm | - | 12mm | 11mm | 15mm |
| **PV4** | 7mm | 2mm | 8mm | 5mm | 10mm | **PV37** | 31mm | 35mm | 41mm | 32mm | 33mm |
| **PV6** | 12mm | 9mm | - | - | 10mm | **PV38** | 14mm | 28mm | 24mm | 21mm | 11mm |
| **PV11** | 10mm | - | 1mm | - | 5mm | **PV38** | 15mm | 10mm | 3mm | - | - |
| **PV12** | 12mm | 8mm | 5mm | - | 4mm | **PV40** | - | 15mm | - | 12mm | - |
| **PV14** | - | - | - | 5mm | 12mm | **PV41** | 5mm | - | 11mm | 8mm | 15mm |
| **PV15** | 15mm | 10mm | 3mm | - | - | **PV45** | 8mm | 3mm | 5mm | 9mm | 15mm |
| **PV16** | - | 5mm | 4mm | 2mm | - | **PV46** | - | 4mm | 9mm | 5mm | 12mm |
| **PV17** | 5mm | - | 12mm | 11mm | 5mm | **PV47** | 7mm | 2mm | 8mm | 5mm | 14mm |
| **PV19** | 13mm | 9mm | - | - | 15mm | **PV48** | - | 3mm | 5mm | 9mm | 15mm |
| **PV20** | 11mm | - | 2mm | - | 6mm | **PV52** | 11mm | - | - | 4mm | 9mm |
| **PV21** | 12mm | 18mm | 15mm | - | 5mm | **PV58** | 21mm | - | - | 4mm | 19mm |
| **PV23** | - | - | 8mm | 5mm | 12mm | **PV59** | - | 5mm | 10mm | 12mm | - |
| **PV24** | 5mm | 10mm | 13mm | - | - | **PV60** | 28mm | 31mm | 35mm | 4mm | 14mm |
| **PV25** | - | 5mm | 4mm | 2mm | - | **PV62** | 13mm | 9mm | - | - | 15mm |
| **PV26** | 5mm | - | 12mm | 11mm | 5mm | **PV63** | 11mm | - | 2mm | - | 6mm |

| Isolates | Diesel | Olive oil | Sunflower | Mustard | Gingelly | Isolates | Diesel | Olive oil | Sunflower | Mustard | ingelly |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **PV64** | 12mm | 18mm | 15mm | - | 5mm | **PV94** | - | - | 9mm | 15mm | 12mm |
| **PV66** | - | - | 8mm | 5mm | 12mm | **PV95** | 7mm | 2mm | 8mm | 15mm | 16mm |
| **PV67** | 5mm | 10mm | 13mm | - | - | | | | | | |
| **PV68** | - | 5mm | 4mm | 2mm | - | | | | | | |
| | 5mm | - | 12mm | 11mm | 5mm | | | | | | |
| **PV71** | 15mm | 10mm | 3mm | - | - | | | | | | |
| | - | - | 9mm | 5mm | 2mm | | | | | | |
| **PV77** | 7mm | 2mm | 18mm | 5mm | 10mm | | | | | | |
| **PV83** | 12mm | 9mm | - | - | 11mm | | | | | | |
| **PV84** | 10mm | - | 11mm | - | 5mm | | | | | | |
| **PV85** | 12mm | 8mm | 5mm | - | 13mm | | | | | | |
| **PV87** | - | - | - | 15mm | 12mm | | | | | | |
| **PV88** | 15mm | 10mm | 13mm | - | 14mm | | | | | | |
| **PV89** | - | 5mm | 4mm | 2mm | - | | | | | | |
| **PV90** | 5mm | - | 12mm | 11mm | 5mm | | | | | | |
| **PV91** | - | - | 9mm | 15mm | 12mm | | | | | | |
| **PV92** | 7mm | 2mm | 8mm | 15mm | 16mm | | | | | | |
| **PV93** | - | 3mm | 5mm | 9mm | 16mm | | | | | | |

Footnote: The yeast isolates without oil displacement activity are omitted from the table

The Phenol-sulfuric test (glycolipids), Biuret test (lipopeptides), and Phosphate test (phospholipids) were performed with cell-free supernatant of PV 37 to identify the type of biosurfactant (Glycolipids, lipopeptides, phospholipids) produced. As the BS was positive for the phenol-sulphuric acid test and negative for biuret and phosphatase it was classified as glycolipid. Most common biosurfactants are of glycolipid nature, rhamnolipids, sophorolipids, and trehalolipids are some of the common glycolipids. Glycolipid biosurfactants have been reported from yeasts such as Candida, Starmerella, Rhodotorula, and Pseudozyma (Jezierska *et al.*, 2018).

The BS was extracted and precipitated under highly acidic conditions. On evaporation of the solvent, the surfactant from potential strain PV37 remained as white colour sediment and this was used for the chemical characterization studies **(Figure 1).**

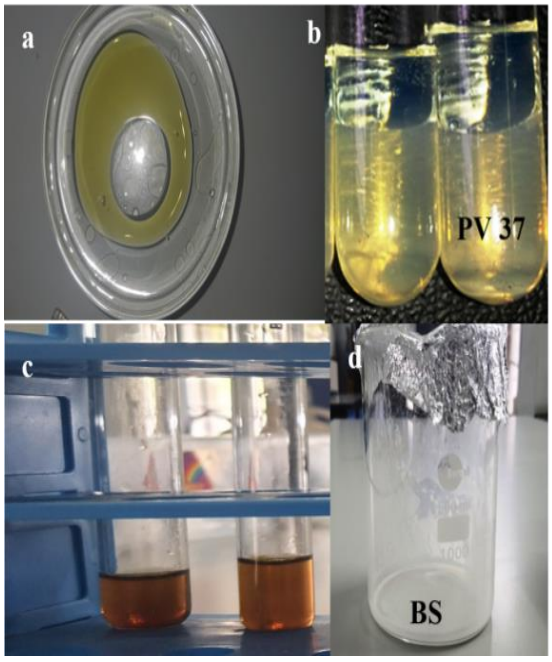


**Figure 1:** Characterization of BS. A, Oil displacement activity (OST); b, Emulsification index; c, Phenol- Sulphuric acid test; d, Purified BS

Low productivity and high production cost of biomolecules are one of the main hurdles to commercialization. So, the challenges faced by the scientist are to evaluate the optimum growth conditions and alternate raw materials for biomolecule production. To obtain insight into yeast growth and BS production, the strain was grown on a sunflower oil medium and its kinetic parameters were evaluated Vijaya *et al.* (2013) too have used low-cost renewable substrates like waste olive oil, palm oil, sunflower oil, etc in their study to reduce production cost.

The samples from the production flasks were retrieved every 24 hrs to measure optical density, the diameter of displacement zone (OST), E24, and BS yield. The results are shown in **Figure 2**. The culture PV 37 entered the log phase of growth after 24 hrs of incubation and reached maximum growth at 120 hrs. Then the culture entered into stationary phase. The BS production began after 48 hrs of incubation and maximum production was noted at 120 hrs of incubation as indicated by an increase in OST, emulsification index and yield. The maximum biosurfactant yield was 2.8 g/L.

The stability of biosurfactants under varying environmental conditions is a major bottleneck in its commercialization. Therefore, in this study, we examined the stability of BS from PV37 under varying temperatures, salinity, and pH **(Figure 3)**.

The BS of PV37 was found to be moderately thermophilic and stable with maximum activity at a temperature range of 30-60º C. Since majority of the industries work at temperatures above at 40 ºC, the significance of thermostable biosurfactants is of extraordinary importance. BS from PV37 retained its stability in the pH range of 6-10. The BS stable under alkaline conditions is ideal for the detergent industry. BS was stable under saline conditions retaining up to 87% activity in the 20% NaCl. Similarly, the yeast strain *Rhodotorula glutinis* exhibited stability under various environmental conditions (Ortansa *et al.*, 2012). The reason for the stability of the BS could be attributed to the fact that the producer strain PV 37 is adapted to the harsh coastal conditions found in the mangrove ecosystem.

TLC analysis of purified BS from PV 37 showed a blue spot with Molish reagent and iodine vapour, indicating the presence of carbohydrate and lipid moieties. Staining of the TLC plate with ninhydrin did not result in any blue colour development, showing that the biosurfactant did not contain any peptide or amino acid. Therefore, these results indicated that the extracted biosurfactant contains a lipid part and carbohydrate part, therefore glycolipid. The Rf value of 0.88 of PV 37 BS is similar to Rf values reported for glycolipids BS in literature (Silva *et al.*, 2010, 2014). A single spot on a TLC plate confirmed its purity.

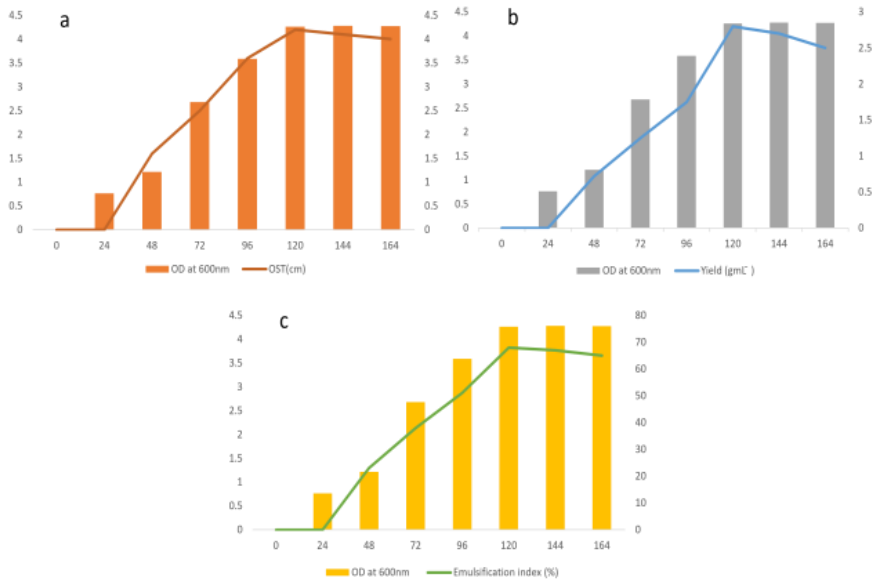


**Figure 2:** Relation between Growth and BS production. A, Growth and OST; b, Growth and yield; c, Growth and emulsification index.

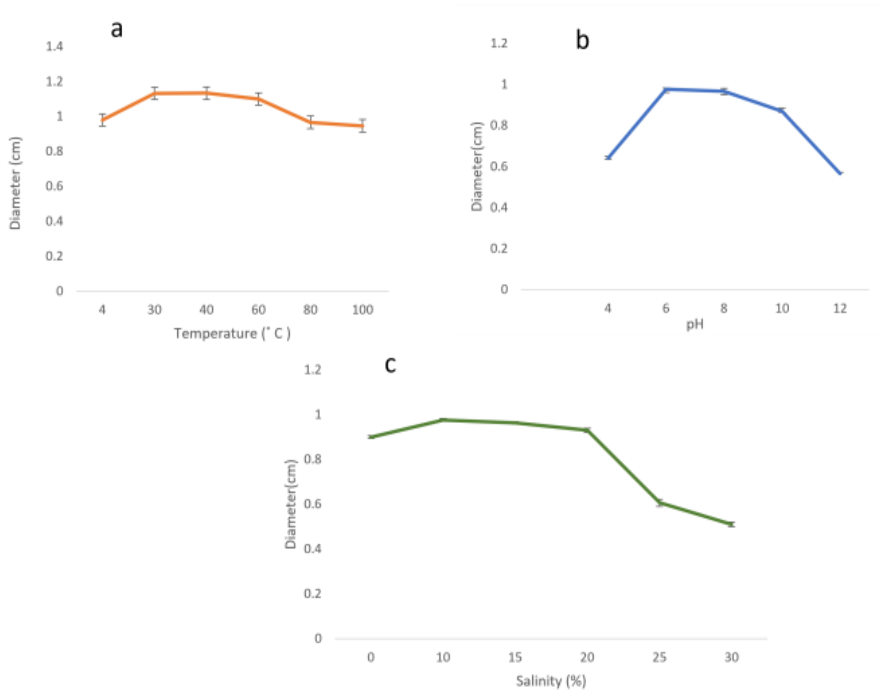


**Figure 3:** Stability of PV37 BS. A, Temperature b, pH; c, Salinity

In FTIR analysis the bands at 3490 and 3352 $cm^{-1}$ indicated the presence of free –OH groups due to H-bonding of polysaccharides and –OH stretching of carboxylic acid groups, respectively. The peak at 2963 $cm^{-1}$ is the stretching vibration of the –CH bond of the respective sugar moiety. The 1673 and 1634 $cm^{-1}$ peaks indicated the C = O stretching in ester groups of lipids and fatty acids. while the deformation vibration of alkyl groups was confirmed by the presence of peaks at 1382 and 1379. The absorption peaks at around 1468 and 1109 $cm^{-1}$ indicated the stretching bands of carbon atoms with hydroxyl groups in the structure of sugar moiety and bands at 1013 and 617 $cm^{-1}$ were associated with the stretching vibrations of glycosidic linkage which confirmed the glycolipid nature of the BS. The FTIR spectra of glycolipid were nearly the same as those reported for other glycolipid BS (Sharma *et al.*, 2015; Hemlata *et al.*, 2015; Singh *et al.*, 2016) **(Figure 4).**

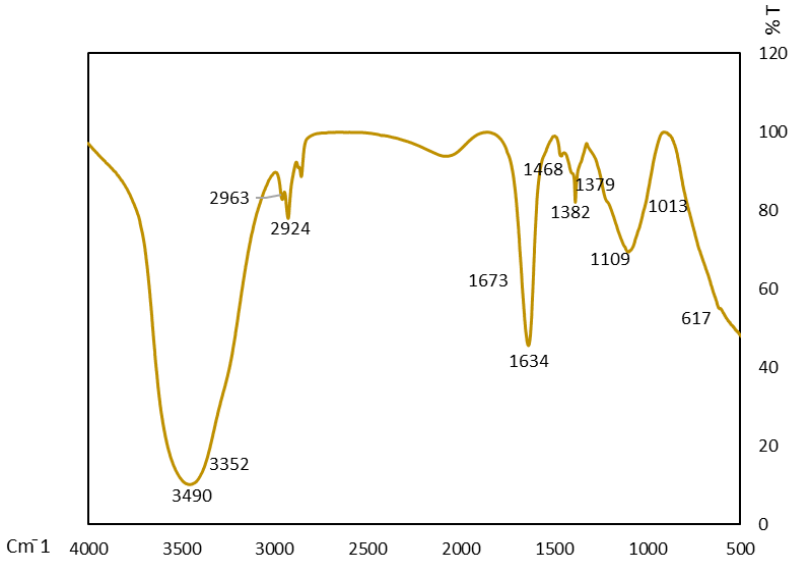


**Figure 4:** FTIR spectra of BS from PV37

The antibacterial activity of the BS against 13 bacterial pathogens with a MAR (multiple antibiotic resistance) indexes greater than 0.2 was analyzed in this current study. The glycolipid from PV 37 more strongly inhibited Gram-negative pathogens than Gram-positive ones. A maximum zone of inhibition was noted in the case of the bacterium *A. hydrophyla* (MTCC 1739) followed by *E. tarda* (MTCC 2400) and *V. cholerae* (MTCC 3906) **(Figure 5).**

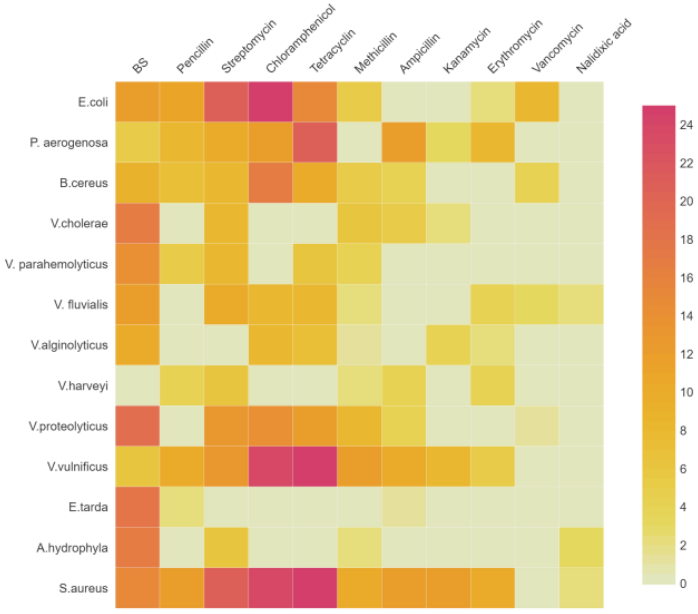


**Figure 5:** Heatmap of the Antibiotic and BS Susceptibility profile of bacterial pathogens

The antibacterial results with current biosurfactant were comparable with those obtained from previous reports, the BS from *Candida parapsilosis* showed significant antibacterial activity against pathogenic Gram positive and Gram-negative organisms (Garg *et al.*, 2018).

In the cell lysis method, the growth inhibition reveals the percentage of cell lysis. The comparison of the absorbance reading of the culture broth (control) with the test showed that the highest percentage of inhibition was noted against *Aeromonas hydrophyla* (90.67%) immediately after the addition of BS at a concentration of 1mg/ml. The inhibition was higher than SDS (Positive control) the commercially used surfactant **(Table 2).**

**Table 2.** Comparison bacterial cell lysis with SDS and BS

| Organism | SDS (%) | BS (%) |
|---|---|---|
| *E. coli* | 48.87 ± 0.23 | 57.30 ± 0.24 |
| *P. aerogenosa* | 32.63 ± 0.12 | 44.51 ± 0.21 |
| *B.cereus* | 23.93 ± 0.25 | 38.24 ± 0.13 |
| *V.cholerae* | 76.99 ± 0.35 | 88.08 ± 0.10 |
| *V. parahemolyticus* | 73.07 ± 0.15 | 86.54 ± 0.10 |
| *V. fluvialis* | 74.23 ± 0.12 | 85.23 ± 0.24 |
| *V.alginolyticus* | 50.18 ± 0.15 | 62.14 ± 0.19 |
| *V.harveyi* | 68.08 ± 0.35 | 86.08 ± 0.17 |
| *V.proteolyticus* | 47.74 ± 0.23 | 83.85 ± 0.28 |
| *V.vulnificus* | 75.51 ± 0.15 | 82.36 ± 0.10 |
| *E.tarda* | 59.41 ± 0.24 | 87.58 ± 0.11 |
| *A.hydrophyla* | 72.66 ± 0.10 | 90.67 ± 0.12 |
| *S.aureus* | 43.18 ± 0.24 | 38.24 ± 0.12 |

The DPPH and ABTS radical scavenging assay have been generally used to study the capability of a compound to act as scavengers or hydrogen donors (Uddin *et al.*, 2008). The glycolipid biosurfactant exhibited effective antioxidant activity in a concentration-dependent manner. The BS from PV37 at a concentration of 100µg/ml exhibited 89% and 97% antioxidant activity by DPPH analysis and ABTS assay respectively **(Figure 6).**

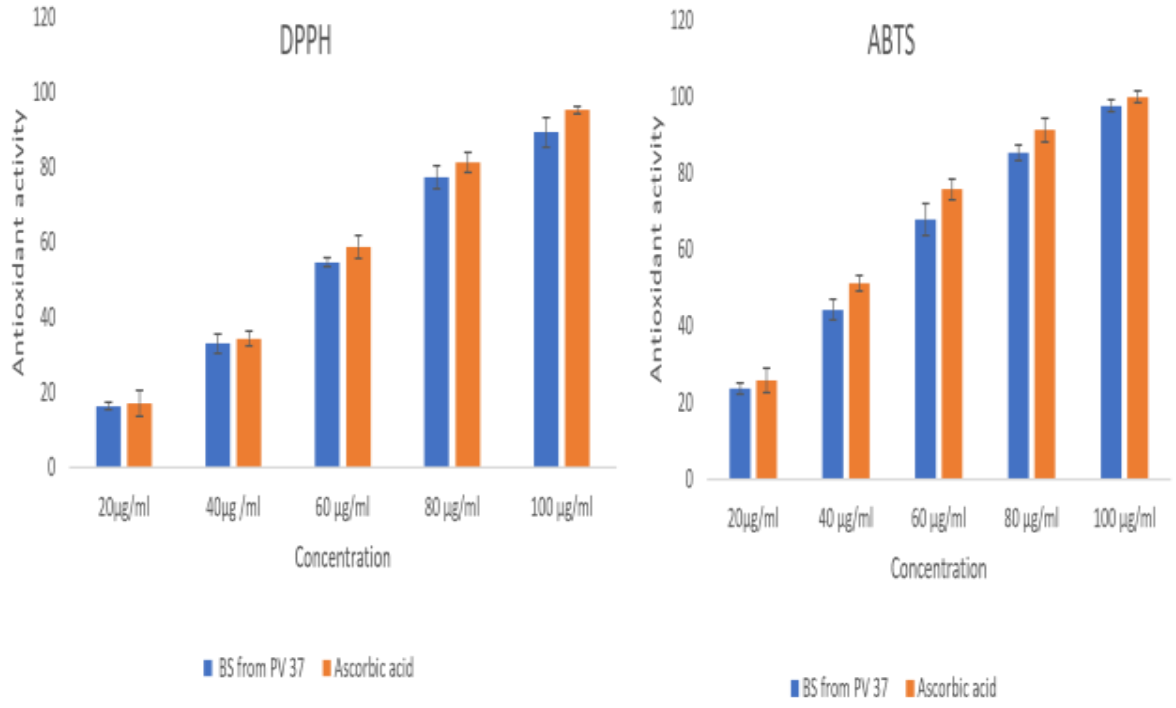


**Figure 6.** Antioxidant activity of BS from PV37

The cytotoxic effects of glycolipid were measured in the MCF7 cell line using the MTT method. MCF7 cells were treated with various concentrations of glycolipid (6.25, 12.5, 25, 50, 100 µg/mL) for 24 h at 37° C **(Figure 7**). The viability rate of MCF7 cells treated with glycolipid is shown in **Figure 8**. Dose-dependant reduction in cell viability was observed in MCF-7 cancer cells administered with different concentrations of the sample.

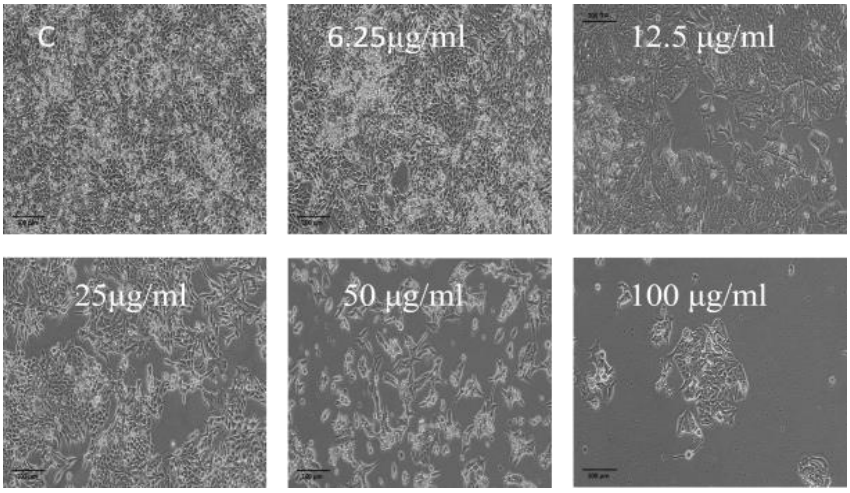


**Figure 7.** Cytotoxic study with BS

The maximum cytotoxicity was observed with 100 µg/ml of the sample. The glycolipid BS showed more significant ($P < 0.01$) cytotoxicity with an LC50 value of 66.59 µg/mL against MCF-7. A 24 hrs exposure showed significant dose-dependent cytotoxic activity. This is a preliminary indication that the tested sample possesses anticancer activity. In the case of Glycolipids, there is a huge variation in their cytotoxicity with di rhamnolipids and mono rhamnolipid from one study to the other (Zhao *et al.*, 2013, Rahimi *et al.*, 2019). The LC50 values could emanate from the interaction between the specific chemical structure of the glycolipid and the membrane biophysical properties of a particular cell type (Rahimi *et al.*, 2019). Adu *et al.* 2022 studied the effects of glycolipid BS on skin cells as well as on malignant melanoma cells compared to healthy human keratinocytes. They found that glycolipid BS prevented the migration of a melanoma cell line, and have antimetastatic potentials.

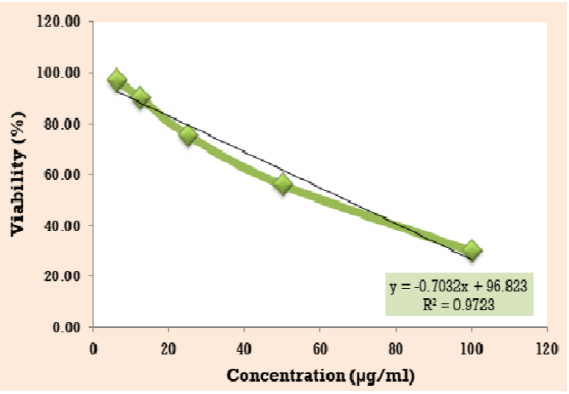


**Figure 8.** Viability rate of MCF 7 cell lines

PV 37 was identified as *Geotrichum candidum* PV 37 by PCR followed by gene sequencing. The results were submitted to and accepted by the National Centre for Biotechnology Information (NCBI) with accession number AC No: ON261370 **(Figure 9)**.

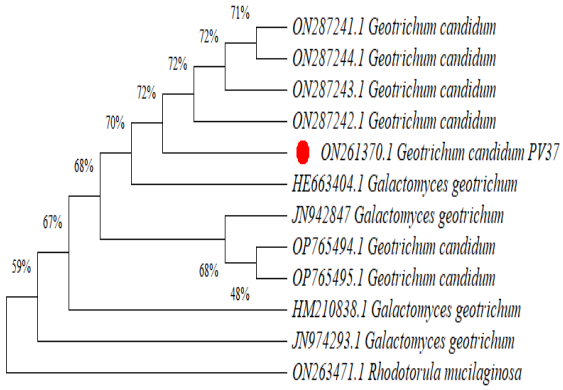


**Figure 9.** Phylogenetic tree

## CONCLUSION

In today's scenario, glycolipid BS are very important due to their non-toxic nature, high biodegradability, low surface tension, and high emulsifying activity. They also possess properties like antimicrobial and antitumor activity at low concentrations. The glycolipid BS from yeast strain *Geotrichum candidum* PV 37 exhibited strong antimicrobial, antioxidant, and anticancer activity, which has high stability at a wide range of pH levels, high temperature and salinity. As this BS is from a GRAS strain with added anticancer biological activity, glycolipids could form a substitute for commercially available chemotherapeutic agents. Although the specific mechanisms are not fully understood. they can be used in green technologies. As BS is active at low concentration and with little impact on the

environment they are ideal candidate to be used in cosmetic, pharmaceutical, food, and health-care industries.

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**CONFLICT OF INTEREST**

No

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