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Antimicrobial and Anti Adhesive Activity of Purified Biosurfactants Produced by *Candida species*

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Abstract: Biosurfactant produced by *Candida tropicalis* and *C. albicans* was first partially purified in a separating funnel using the solvent dichloromethane. Then the partially purified biosurfactant was analyzed by High performance liquid chromatography. The chemical structure of the biosurfactants was determined by Infra Red Spectroscopy (IF-TR). The results showed the presence of hydroxyl, amino, olefin and carboxylic groups present in both type of biosurfactants produced by *C. tropicalis* and *C. albicans*. *C. tropicalis* biosurfactants has ketone and ester groups, where as, *C. albicans* biosurfactants has carbonyl, aromatic and nitro groups. In this current study, the biofilm formation assay was performed with different types of clinical and urinary isolates (*Bacillus, Candida albicans, Citrobacter, Escherichia coli. Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa., Salmonella and Staphylococcus aureus). Among the isolates used in the study, Proteus mirabilis, S. aureus, P. aeruginosa, Bacillus and Citrobacter, were produced Biofilm where as Salmonella, E. coli, Candida albicans and K. pneumoniae were negative for biofilm formation.* In the present study, comparatively biosurfactant synthesized by *C. tropicalis* have the highest ability of antimicrobial activity than *C. albicans* against all microbial cultures. In this study, the anti-adhesive activity of biosurfactants was evaluated against a variety of urinary and clinical pathogens. Adherence of cells was highly reduced in the biosurfactants synthesized by *C. tropicalis* than the biosurfactants synthesized by *C. albicans*. Moreover, the biosurfactant showed anti-adhesive activity against most of the microorganisms tested. The results obtained in this work showed that the biosurfactant from *C. tropicalis* is a potential antimicrobial and anti-adhesive agent for several biomedical applications.

Key words: Biosurfactant · Antimicrobial · Antiadhesive · Biofilm · Purification · Adherence

INTRODUCTION

The surface active molecules of microbial origin are referred to as biosurfactants. Unlike chemical surfactants, which are mostly derived from petroleum food stock, these molecules can be produced by microbial fermentation process using cheaper agro based substrates and waste materials [1-4]. Biosurfactant have advantages over their synthetic chemical counterparts because of their biodegradability and reduced toxicity, availability from cheap raw materials, biocompatibility and the effectiveness at extreme temperature, pH and salinity. Biosurfactants have found possible applications in biomedical fields [5].

Biosurfactants are amphihilic biological compounds that are produced extracellularly or intracellularly by a wide variety of microorganisms, which include bacteria, yeasts and filamentous fungi [6-8]. These polarities render surfactants capable of reducing surface and interfacial tension and forming micro emulsion where hydrocarbon can be solubilize in water or where water can solubilize in hydrocarbons. Such characteristics confer excellent detergency, emulsifying, forming and dispersing traits [9]. It has both lipophilic and hydrophilic structural moieties in its molecule. Most microbial surfactants are complex molecules, comprising different structures that include lipopeptides, glycolipids, polysaccharide-protein complex, fatty acids & phospholipids [10].

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Biosurfactant producing microorganisms were naturally present in the oil contaminated soil. Oil contaminated environment contain large amount of hydrocarbons. Microorganisms' exhibit emulsifying activity by producing biosurfactants often mineralizing them (or) converting them into harmless products. They are more active and less toxic than chemical surfactants which are difficult to remove or degrade from the environment. They can be used in handling industrial emulsions, control of oil spills, biodegradation and detoxification of industrial effluents and in bioremediation of contaminated soil [11].

The incidence of bacterial infections in patients with urinary catheters is approximately 5 to 10% per day, with virtually all patients who undergo long-term catheterization (28 days) becoming infected [12]. Many bacterial species colonize indwelling catheters, growing as biofilm communities embedded in a gel-like polysaccharide matrix and induce complications in patients' care. The most common organisms associated with CAUTI are *Escherichia coli* (21.4%), *Candida sp.,* (21%), *Enterococcus* sp., (14.9%), *Pseudomonas aeruginosa* (10%), *Klebsiella pneumonia* (7.7%) and *Enterobacter sp.,* (4.1%) [13]. All types of catheter, including those coated in antimicrobial agents, are vulnerable to encrustation by these biofilms and there is a clear clinical need to develop prevention strategies. Bacterial cells in the biofilm mode of growth are resistant to antibiotics and evidence indicates that treatment of symptomatic urinary tract infection is more effective if biofilm laden catheters are changed before antibiotic treatment is initiated.

Biosurfactants have been found to inhibit the adhesion of pathogenic organisms to solid surfaces or to infection sites; thus, prior adhesion of biosurfactants to solid surfaces might constitute a new and effective means of combating colonization by pathogenic microorganisms [14]. Pre-coating vinyl urethral catheters by running the surfactin solution through them before inoculation with media resulted in a decrease in the amount of biofilm formed by *Salmonella typhimurium, Salmonella enterica, E. coli* and *Proteus mirabilis* [15].

The present study revealed the antimicrobial and anti adhesive activity of purified biosurfactants from *Candida* species. The present investigation concludes that the more activity of biosurfactants identified in *Candida tropicalis* than the *Candida albicans*. Also the biosurfactants synthesized by *C.tropicalis* showed highest activity of antimicrobial and anti-adhesive nature against microbial pathogens in urinary catheters.

MATERIALS AND METHODS

In the present study, *Aspergillus niger, Bacillus, Candida albicans, Candida tropicalis, Klebsiella* and *Pseudomonas aeruginosa,* were isolated from the collected petroleum contaminated soil samples. Screening of biosurfactant producing potential strain was done using oil spreading method [16], oil collapse [17], emulsification method [18] and MATH assay method [19]. The potential strain for biosurfactant production was screened and the activity was tested further.

Optimization Studies [20]: To find the optimum conditions for biosurfactant production, the production media were maintained at various incubation periods (3, 6, 9, 12 and 15 days), temperature (10, 20, 30, 40 and 50°C), pH (2, 4, 6, 8 and 10) using 1N NaOH or 1N HCl and the substrate concentration (0, 0.5, 1.0, 1.5, 2.0 and 2.5% w/v) for both *C. tropicalis* and *C. albicans*. Both cultures were inoculated in a 50mL of mineral salt medium and incubated. After the incubation period, cultures were centrifuged at 10,000 rpm for 10 minutes, supernatant was discarded and the pellets were taken and mixed with 5 mL of PUM buffer then the OD values were measured at 600nm.

Production of Intracellular Biosurfactant [21]: For the production of biosurfactant 1000mL of mineral salt medium was prepared with optimum pH and then the substrate with optimum concentration was added into the broth with both cultures of *C. tropicalis* and *C. albicans* and was incubated in an optimum temperature and incubation period. After incubation, the broth cultures were centrifuged at 10,000 rpm for 10 minutes. Then the pellets were washed with PUM buffer and then used for further purification.

Purification of Biosurfactant

***Partial purification* [22, 23]:** The pellets were resuspended in a 40mL of dichloromethane in a separating funnel and shaken vigorously; allowed surfactant was recovered in the organic layer at the top. The extraction was performed twice and the organic layers were pooled and evaporated. The organic layers were collected in separate conical flasks and the partially purified biosurfactant were given to HPLC analysis.

HPLC Analysis [23, 24]: Isolated biosurfactant was analyzed by High Performance Liquid Chromatography equipped with a C 18 column (5µm). The mobile phase consisted of 20% trifluoroacetic acid and 80% acetonitrile. The absorbance of the eluent was monitored at 205nm and the flow rate was 1.20 and 0.50 mL/min.

Fourier Transform Infrared Spectroscopy [25,26]: FT-IR spectroscopy can be used to elucidate the chemical structures of some components in an unknown mixture by identifying the types of chemical bonds or the functional groups present in their chemical structures. For FT-IR analysis potassium bromide (KBr) was crushed and pressed to obtain translucent pellets and 20 µL of liquid samples were dropped onto the pellets and were placed into the FT-IR analyzer.

Isolation of Urinary Clinical Pathogens: Clinically important urinary pathogens were collected from hospitals nearby around Coimbatore. Organisms were isolated and identified by the preliminary tests [27].

Biofilm Formation Assay [28]

Test Tube Method: Sterile 5mL of Mueller Hinton broth was taken in a sterile test tube and then inoculate with test organisms (*Bacillus, Candida albicans, Citrobacter, E. coli., Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella and Staphylococcus aureus*). Maintain 1 tube as a control and incubate at 37°C for 24 hours. After 24 hrs of incubation broth was discarded and washed with 0.5 M of Phosphate Buffer Saline (PBS). After washing with saline the tubes were stained with 1% crystal violet dye solution. The excess stain was washed off and removed. Dry the tubes in inverted position for a while.

Antimicrobial Activity of Biosurfactants [29]: Mueller Hinton agar was prepared and poured in a sterile petriplate. The plates were allowed to solidify. After solidification the urinary isolates and some clinical isolates (*Bacillus, Candida albicans, Citrobacter, E. coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella and Staphylococcus aureus*) were swabbed with sterile cotton swabs. Then 30 µL of purified biosurfactants, synthesized from *C. albicans* and *C. tropicalis* were dropped onto 10 mm in diameter sterile discs. The plates were incubated at 37°C for 24 hours. After incubation the results were observed.

Anti-Adhesive Activity of Biosurfactants [30, 25]: Biosurfactants have been found to inhibit the adhesion of pathogenic organisms to solid surfaces or to infection sites; thus, prior adhesion of biosurfactants to solid surfaces might constitute a new and effective means of combating colonization by pathogenic microorganisms.

Nutrient broth was prepared. And 5 mL of broth was taken in each sterile test tube. Three sets of tubes were made; urinary catheters with test cultures were inoculated in a one set of tubes and in another two sets contain urinary catheters were precoated with biosurfactant synthesized from *C. albicans* & *C. tropicalis,* then allowed to dry the catheters, after that the precoated catheters were dipped into test cultures. The tubes were shaken well; in three sets of tubes in each set one tube maintain as a control. In the first set contain only sterile urinary catheters, in second tube contain sterile catheter with biosurfactant is synthesized from *C. albicans* and in the third set of tube contain sterile catheter with biosurfactant is synthesized from *C. tropicalis.* Then all the tubes were incubated at 37°C for 24 hours. After incubation the OD values were measured at 600 nm absorbance. Sterile broth was used as a blank.

RESULTS AND DISCUSSION

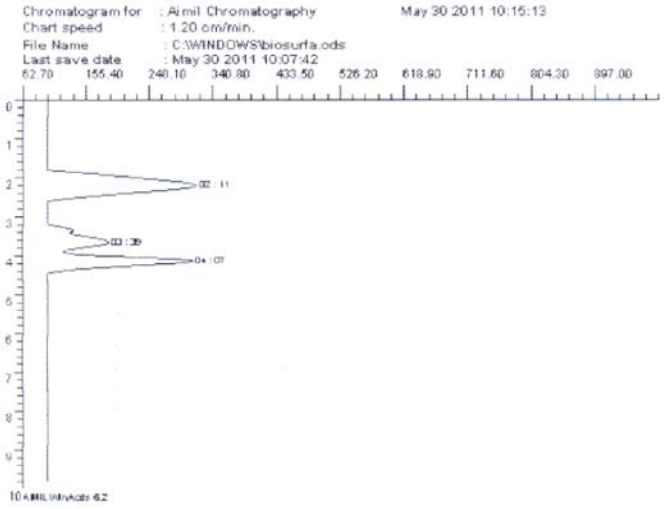
In the present study petroleum contaminated soil samples were collected and the organisms *Klebsiella, Bacillus, Aspergillus niger, Candida tropicalis, Candida albicans* and *Pseudomonas aeruginosa* were isolated from oil contaminated soils. Screening of biosurfactant producing potential strain was done using oil spreading method, oil collapse method; emulsification method and MATH assay method. Using preliminary tests, the potential strain was identified as *Pseudomonas aeruginosa, Candida tropicalis* and *Candida albicans*. In oil spreading method, the organisms were produced maximum zone. High concentration of both the extra cellular biosurfactant was produced by *P. aeruginosa* and intracellular biosurfactant was produced by *C. tropicalis* and *C. albicans* using emulsification method. In the present study, the MATH assay and statistical analysis revealed that *C. tropicalis* and *C. albicans* have higher hydrophobicity than *P. aeruginosa.* Optimization studies were carried out for biosurfactant production using different parameters like incubation period, temperature, pH and substrate concentration with selected *Candida* species (*C. tropicalis* and *C. albicans*).

Table 1: HPLC analyses of the purified biosurfactant biosynthesized by *Candida albicans* and *Candida tropicalis* cultivated on diesel oil.

S.No	Name	Peak symbol	RT time min:sec	Height mm & mV	Area mm & mV-sec	Amount % Area
1	Standard biosurfactant	A	3.195	4850 (mm)	54062	33.245
2	*Candida albicans*	B	04:07	195.080 (mV)	2783.986 (mV)	29.442
3	*Candida tropicalis*	C	04:19	116.362 (mV)	1482.172	7.305

Total Area of *Candida albicans* is 9455.902 mV-secs.

Total Area of *Candida tropicalis* is 20288.949 mV-secs.



Peak No	RT min:sec	Height mV	Area mV-sec	Amount % Area	Name
1	02 : 11	217.264	5732.794	60.627	*Candida albicans*
2	03 : 39	60.675	939.122	9.932	
3	04 : 07	195.080	2783.986	29.442	

Total Area : 9455.902 mV-sec

Fig. 1: HPLC analysis *C. albicans*

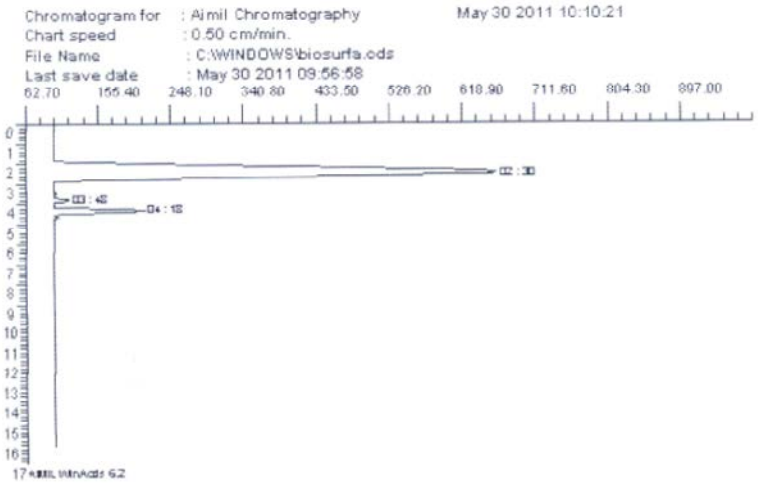
In the present study the biosurfactant was produced in optimum parameters like incubation period, temperature, pH and substrate concentration. The maximum production in 6th day, 40°C and 6 respectively for both *C. tropicalis* and *C. albicans*, the substrate concentration was 1.5% *for C. tropicalis* and 2.0% for *C. albicans*. The total quantity of production of biosurfactant was 1.71% for *C. albicans* and 0.91% biosurfactant was produced by *C. tropicalis*.

Optimization studies were carried out for maximum biomass production. Incubation period seems to be the important factor for the biomass production and maximum growth was observed at 72h, growth in different pH values showed maximum at pH 7 and minimum at 10, maximum growth at temperature 35°C and minimum at 25°C, higher growth was observed at 0.3% salinity and minimum at 1.5% and maximum growth using glucose and minimum using glycerol carbon source was observed [31].

Purification of Biosurfactant: Purification of biosurfactant was done by two methods. Biosurfactant was first partially purified by separating funnel; dichloromethane was used as a solvent for purification. Then the partially purified biosurfactant was analyzed by High performance liquid chromatography. The values are shown in Table 1.

The purified SAC of *B. subtilis-27* was detected three peaks A, B and C by using HPLC analysis technique, the highest SAC was presented at peak C. The three peaks appeared at retention time 3.925, 4.533 and 5.664 (min). The highest activity presented at peak C concentration (82.531%). In HPLC, three major peaks were observed between 5.6 and 10.9 min which showed the activity in *Bacillus subtilis* B20 [32].

Fourier Transforms Infrared Spectroscopy: In the present study the both types of biosurfactant were synthesized by *C. tropicalis* and *C. albicans* have many



Peak No	RT min:sec	Height mV	Area mV-sec	Amount % Area	Name
1	02 : 30	564.851	18634.846	91.847	*Candida tropicalis*
2	03 : 49	19.013	171.931	0.847	
3	04 : 19	116.362	1482.172	7.305	

Total Area : 20288.949 mV-sec

Fig. 2: *HPLC* analysis *C. tropicalis*

functional groups similarly. That is alcoholic (O-H) ranges from 3668 to 3479, amine (C-N) ranges from 1315 to 1338, alkene group ((-C=C-) varies from 1631 to 1651, alkane and acid groups were present in both types of biosurfactants. *C.tropicalis* biosurfactants has ketone and ester groups; where as, *C.albicans* biosurfactants has carbonyl, aromatic and nitro compound (Table 2 & 3, Figure 3, 4).

The IR spectrum of *B. subtilis* NRRL B-94 biosurfactant showed strong bands, indicating the presence of a peptide component at 3360 cm-1 resulting from the N-H stretching mode and at 1637 cm-1 (stretching mode of the CO-N band). The bands at 2925 – 2855 cm^{-1} and at 1458 - 1376 cm-1 reflect aliphatic chains (CH3 – CH2 -), while the band at 1741 cm-1 refer to the presence of ester carbonyl group [33].

For the biofilm formation assay 12 different types of clinical isolates and urinary isolates (*Salmonella*, *Proteus mirabilis, Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus*, *Escherichia coli*, *Candida albicans, Klebsiella pneumoniae* and *Citrobacter*) were used. Among the isolates used in the study, *Proteus mirabilis, S. aureus*, *P. aeruginosa*, *Bacillus* and *Citrobacter,* were produced biofilms where as *Salmonella*, *E. coli, Candida* and *K. pneumoniae,* were negative for biofilm formation. The results were shown in (Table 4, Figure 5).

The biofilm forming capacity of the isolate *Brevibacterium aureum* MSA13 was analyzed in the solid surface of glass tubes. The isolate *B. aureum* MSA13 showed the maximum binding capacity on both surface of glass tubes and polystyrene plates [34].

The antimicrobial activity of biosurfactants was evaluated against a variety of bacterial and a single yeast cultures. Both types of biosurfactants were tested. The biosurfactant synthesized by *C.tropicalis* have the highest ability of antimicrobial activity against all microbial cultures. The biosurfactant synthesized by *C. albicans* have the ability to inhibit only some of the organisms like *Proteus mirabilis, E.coli, Candida albicans* and *Klebsiella pneumoniae.* Against *Salmonella,* it shows only intermediate activity. Then the remaining organisms *Pseudomonas aeruginosa, Bacillus, S. aureus* and *Citrobacter,* were resistant for the biosurfactants. The results were shown in (Table 5, Figure 6, 7, 8).

The lipopeptide biosurfactant from *Bacillus natto* TK-1 exhibited both antibacterial and antifungal activities [29].

Table 2: Shows FT-IR results for biosurfactant synthesized by *C. tropicalis*

Wave Numbers (Cm^{-1})	Bond (Functional group)	Mode (Type of vibration)
3479	Alcohol (O-H)	Stretch, free
3444	Alcohol (O-H)	Stretch, free
2430	Alkene (-C=C-)	Stretch
2384	Alkene (-C=C-)	Stretch
2303	Alkene (-C=C-)	Stretch
1967	Ketone (α, β-unsaturated)	Stretch
1651	Alkene (C=C)	Stretch
1458	Alkane (-C-H)	Bending
1396	Alkane (-C-H)	Bending
1361	Alkane (-C-H)	Bending
1315	Amine (C-N)	Stretch
1288	Ester (C-O)	Stretch
1273	Ester (C-O)	Stretch
1242	Ester (C-O), Acid (C-O)	Stretch
987	Alkane (-C-H)	Stretch
937	Alkane (-C-H)	Bending
852	Alkane (-C-H)	Bending
705	Alkane (-C-H)	Bending
520	Alkane (-C-H)	Bending

Table 3: Shows FT-IR results for biosurfactant synthesized by *Candida albicans*

Wave Numbers (Cm^{-1})	Bond (Functional group)	Mode (Type of vibration)
3668	Alcohol (O-H)	Stretch, free
3645	Alcohol (O-H)	Stretch, free
3583	Alcohol (O-H)	Stretch, free
3479	Alcohol (O-H), Amine (N-H)	Stretch, H-bonded
3441	Alcohol (O-H), Amine (N-H)	Stretch, H-bonded
3417	Alcohol (O-H), Amine (N-H)	Stretch, H-bonded
2542	Acid (O-H)	Stretch
1789	Carbonyl (C=O)	Stretch
1631	Alkene (C=C), Alkane (-C-H)	Stretch, Bending
1454	Aromatic (C=C), Alkane(-C-H)	Stretch, Bending
1392	Aromatic (C=C)	Stretch
1361	Nitrocompounds (N-O)	Stretch
1338	Amine (C-N)	Stretch
1315	Amine (C-N)	Stretch
1269	Acid (C-O)	Stretch
1091	Alcohol (C-O)	Stretch
987	Alkene (=C-H)	Bending
937	Alkene (=C-H)	Bending
804	Alkene (=C-H)	Bending
686	Alkene (=C-H)	Bending
528	Alkene (=C-H)	Bending

Table 4: Biofilm formation of various clinical and urinary isolates

S.No	Microorganisms	Biofilm formation
1	*Salmonella*	Negative
2	*Proteus mirabilis*	Positive
3	*S. aureus*	Positive
4	*P. aeruginosa*	Positive
5	*Bacillus*	Positive
6	*E. coli*	Negative
7	*Candida albicans*	Negative
8	*Citrobacter*	Positive
9	*Klebsiella pneumoniae*	Negative

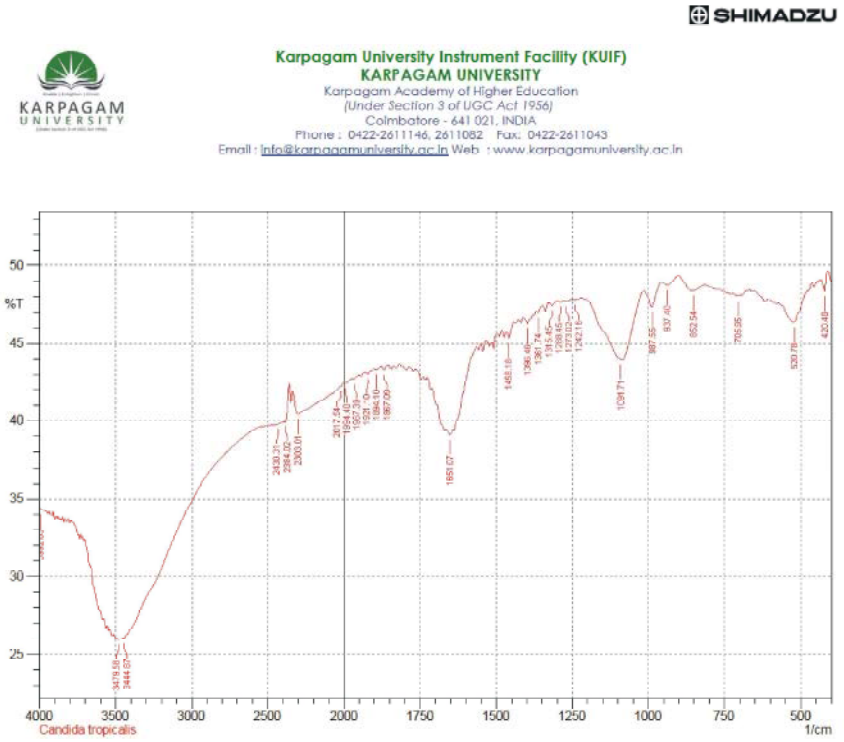
Table 5: Antimicrobial activity of biosurfactants

S.No	Organisms	Antimicrobial activity	
		Biosurfactant synthesized by *C.tropicalis*	Biosurfactant synthesized by *C.albicans*
1	*P. aeruginosa*	++	-
2	*Citrobacter*	++	-
3	*K. pneumoniae*	+	+
4	*Bacillus*	+	-
5	*E. coli*	+	+
6	*Proteus mirabilis*	+	+
7	*S. aureus*	+	-
8	*Salmonella*	+	±
9	*Candida albicans*	+	+

+ → Positive

± → Intermediate

- → Negative



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	420.48	48.335	1.11	432.05	412.77	5.981	0.081
2	520.78	46.336	1.683	570.93	478.35	30.237	0.724
3	705.95	48.016	0.234	721.38	682.8	12.252	0.052
4	852.54	48.372	0.624	898.83	817.82	25.344	0.261
5	937.4	48.731	0.316	952.84	902.69	15.544	0.071
6	987.55	47.319	1.318	1014.56	956.69	18.39	0.296
7	1091.71	43.982	4.195	1215.15	1018.41	66.422	3.924
8	1242.16	47.791	0.041	1249.67	1226.73	7.413	0.003
9	1273.02	47.692	0.062	1276.88	1249.87	8.667	0.007
10	1288.45	47.646	0.084	1300.02	1280.73	6.204	0.009
11	1315.45	47.419	0.232	1327.03	1303.88	7.474	0.024
12	1361.74	46.981	0.231	1369.46	1350.17	6.294	0.016
13	1396.46	46.255	0.453	1408.04	1381.03	8.971	0.051
14	1458.18	45.332	0.653	1465.9	1442.75	7.839	0.062
15	1651.07	39.137	0.946	1662.64	1566.2	36.73	0.572
16	1867.09	43.318	0.25	1874.81	1855.52	6.978	0.023
17	1894.1	43.349	0.065	1897.95	1878.67	6.984	0.008
18	1921.1	43.04	0.193	1928.82	1901.81	9.841	0.024
19	1967.39	42.708	0.079	1971.25	1951.96	7.099	0.004
20	1994.4	42.444	0.085	1998.25	1975.11	8.579	0.01
21	2017.54	42.211	0.098	2025.26	2002.11	8.64	0.008

Fig. 3: FT-IR analysis of *C. tropicalis*

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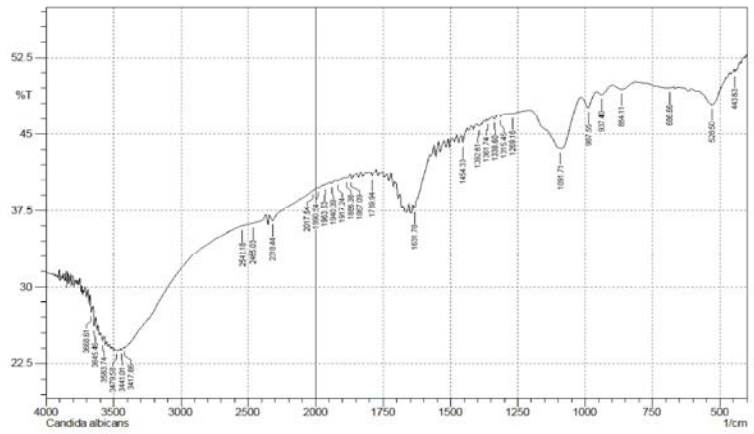

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	443.83	51.049	0.38	447.49	428.2	5.589	0.036
2	528.5	47.798	2.167	578.64	462.92	35.802	1.077
3	686.66	49.474	0.06	709.8	678.94	9.425	0.01
4	864.11	49.349	0.547	898.83	817.82	24.657	0.217
5	937.4	48.833	0.493	956.69	902.69	16.639	0.122
6	987.55	47.545	1.303	1014.56	960.55	17.077	0.28
7	1091.71	43.596	4.502	1203.58	1018.41	63.158	4.093
8	1269.16	46.948	0.07	1276.88	1226.73	16.401	0.018
9	1315.45	46.726	0.101	1319.31	1300.02	6.357	0.008
10	1338.6	46.458	0.266	1342.46	1323.17	6.384	0.02
11	1361.74	46.302	0.195	1365.6	1346.31	6.419	0.016
12	1392.61	45.736	0.327	1400.32	1377.17	7.813	0.04
13	1454.33	44.146	0.844	1456.18	1438.9	6.702	0.067
14	1631.78	37.746	0.859	1639.49	1562.34	30.037	0.204
15	1789.94	40.932	0.36	1801.51	1778.37	8.917	0.026
16	1867.05	40.684	0.411	1870.95	1851.66	7.493	0.044
17	1886.38	40.673	0.163	1894.1	1874.81	7.512	0.018
18	1917.24	40.388	0.188	1924.96	1897.95	10.588	0.026
19	1940.39	40.152	0.179	1944.25	1924.96	7.602	0.015
20	1963.53	39.977	0.13	1971.25	1948.1	9.183	0.015
21	1990.54	39.721	0.104	1994.4	1971.25	9.246	0.016

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22	2017.54	39.48	0.043	2021.4	1998.25	9.314	0.008
23	2318.44	36.522	0.696	2341.58	2021.4	134.375	0.766
24	2465.03	36.262	0.033	2468.88	2372.44	42.207	0.21
25	2542.18	36.025	0.013	2546.04	2476.6	30.685	0.003
26	3417.86	24.031	0.08	3421.72	2549.89	444.459	0.014
27	3441.01	23.824	0.146	3446.72	3429.58	14.379	0.031
28	3479.58	23.747	0.107	3487.3	3468.01	12.028	0.019
29	3583.74	24.733	0.586	3599.17	3572.17	16.209	0.099
30	3645.46	26.165	1.495	3653.18	3633.89	11.027	0.194
31	3668.61	27.521	1.279	3676.32	3657.04	10.704	0.204

Comment;
Candida albicans

Date/Time; 6/15/2011 1:19:14 PM
No. of Scans; 45
Resolution; 8 [1/cm]
Apodization; Happ-Genzel
User; Administrator

Fig. 4: FT-IR analysis of *C. albicans*

1 2 3 4 5 6

Fig. 5: Biofilm formation tube test method
Tube 1: *Pseudomonas aeruginosa*, 2: *Proteus mirabilis,* 3: *Staphylococcus aureus*, 4: *Bacillus,* 5: *Citrobacter* and 6: Control

Table 6: Anti-adhesive activity of biosurfactants

S.No	Microorganisms	Reduction in adherence (%)		
		Without biosurfactant	Biosurfactant synthesized by *C. tropicalis*	Biosurfactant synthesized by *C. albicans*
1	*Pseudomonas aeruginosa*	0.696	0.475	0.920
2	*Citobacter*	0.109	0.002	0.335
3	*Proteus mirabilis*	0.700	0.202	0.640
4	*K. pneumoniae*	0.746	0.630	0.630
5	*Bacillus*	0.084	0.050	0.113
6	*Staphylococcus aureus*	0.205	0.156	0.354
7	*E. coli*	0.780	0.452	0.600
8	*Candida albicans*	0.374	0.122	0.335

Missing

Fig. 6: Antimicrobial activity of biosurfactants *against Citrobacter, E. coli* and *Salmonella*

Fig. 7: Antimicrobial activity of biosurfactants *against Proteus mirabilis, P. aeruginosa* and *K. pneumoniae*

Fig. 8: Antimicrobial activity of biosurfactants *against Candida, S. aureus* and *Bacillus.*

The anti-adhesive activity of biosurfactants was evaluated against a variety of urinary and clinical pathogens. The urinary catheters were inoculated with different types of bacteria and yeast like *Candida.* In this more percentage of cells were adhere to the catheter. After that the catheters were precoated with biosurfactants and again inoculated with all cultures, now the results were showed that the percentage of adherence of cells was reduced. Adherence of cells was highly reduced in the biosurfactants synthesized by *C. tropicalis* than the biosurfactants synthesized by *C. albicans*. The results were shown in Table 6.

In one previous study, a biosurfactants of *P. fluorescens* was found to inhibit the adhesion of *Listeria monocytogenes* LO28 to poly tetra fluoro ethylene and stainless steel surfaces [35].

Petroleum related industry was found to be one of the industries that have a greater potential to produce biosurfactants by a variety of microorganisms [36]. Interest in the production of biosurfactants has steadily increased. However, large-scale production of these molecules has not been realized because of low yields in production processes and high recovery and purification costs. In future, due to its potentiality, some practical approaches that have to be adopted to make the biosurfactant production process economically attractive. Biosurfactants have been found to inhibit the adhesion of pathogenic organism to solid surfaces or to the infection sites. Thus, prior adhesion of biosurfactants to solid surfaces might constitute a new and effective means of preventing biofilm formation by pathogenic microorganisms. A number of technologies have been developed to prevent catheter -associated biofilm formation. Antimicrobial agents are effective against planktonic bacteria and appear to clear mucosal surfaces of adherent bacterial micro colonies but frequently fail to eradicate bacterial Biofilm on urological devices. Since the method of impregnating catheter material with classical antimicrobial agents are not universally effective,

there is a need for more potent and more sophisticated approaches for the prevention of catheter-associated biofilm formation.

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