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Isolation and partial characterization of a biosurfactant produced by *Pseudomonas aeruginosa PAVIJ* from contaminated soil.

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ABSTRACT

Biosurfactant compounds are produced by microorganisms. These isolates reduced surface tension both in aqueous solutions and hydrocarbon mixtures. The objective of this study was to isolate and characterize the surface active components from the crude biosurfactant produced by *Pseudomonas aeruginosa PAVIJ* from contaminated soil. This organism was grown on four different carbon sources (palm oil, coconut oil, honge oil and castor oil). To confirm the ability of isolates in biosurfactant production, different screening methods including blood hemolysis, emulsification, bacterial adherence test for hydrocarbon (BATH) assay, determination of surface tension, drop-collapse, cetyl tri ammonium bromide (CTAB) and methylene blue reduction assay were assessed. The fraction rich in glycolipids was obtained by the fractionation of crude biosurfactant using solid phase extraction and further indentified using thin layer chromatography and confirmed through High performance liquid chromatography. Moreover, this glycolipid rich fractions was found to be antimicrobial agent against several bacterial strains isolated from clinical samples of infected patients. Further purification steps should be carefully analyzed as each purification step will increase the costs and decreases the amounts of biosurfactants recovered.

Keywords: glycolipids, screening assays, surface tension, thin layer chromatography.

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INTRODUCTION

Surfactants are amphiphilic molecules with both hydrophilic and hydrophobic regions attributing towards decrease in surface tension by the formation of aggregates at interfaces between fluids of different polarities. Biosurfactants are a leading group of valuable microbial natural products with unique biochemical properties which have been applied in various areas like detergency, emulsification, adhesion, coatings, wetting, foaming, soil and water remediation, paints, chromatographic separation, medicine, agriculture, cosmetics etc [1,2]. Almost all surfactants currently in use are chemically derived from petroleum and are synthetic in nature exhibiting low rate of biodegradation and high potential to aquatic toxicity. For these reasons, biosurfactants are seen to be a promising alternative with better industrial application. Microorganisms like bacteria, fungi and yeast produce large number of surface metabolites with varied chemical structures and properties [1,3,4].

The interest in biosurfactant has been steadily increasing in recent years due to the possibility of their production through fermentation and their potential applications in such areas as the environmental protection. *Pseudomonas* species form the second largest group of bacteria producing biosurfactants. Many strains of *Pseudomonas* have been reported to produce glycolipids, especially rhamnolipids. Besides rhamnolipids *Pseudomonas* strain such as *Pseudomonas* sp. MIS38 have also been reported to produce arthrofactin, a lipopeptide type of biosurfactant. Other biosurfactants produced by *Pseudomonas* include viscosin produced by *Pseudomonas fluorescens*, putisolvin produced by *Pseudomonas putida* and amphisin produced by *Pseudomonas* sp. DSS73. Rhamnolipid is a glycolipid biosurfactant produced by many strains of *Pseudomonas* [5].

The Biosurfactants produced by the microorganism exhibits emulsifying activity to utilize hydrocarbons as a sole carbon source and to convert them to harmless products [6].The organisms carry out biosurfactant production when grown either on insoluble substrates (such as hydrocarbons, oils and waxes) or on soluble ones (carbohydrates). Hence the isolation of microbial strains capable of biosurfactant production by using these substrates is of interest in commercial production. Kerosene, petrol, diesel, oils are examples of alternative substrates to produce various biosurfactants like glycolipids-rhamnolipids, lipopeptides and surfactins by different species of *Pseudomonas, Bacillus, Serratia, Candida* etc [7,8]. Aim of this work is to isolate bacterial strain from petrochemical waste soil and screen for biosurfactant production using different screening methods. The potential for the degradation of diesel by the bacteria was studied and efforts to partially purify the biosurfactant was also carried out.

MATERIALS AND METHODS

Isolation of *Pseudomonas aeruginosa PAVIJ* from petrochemical site

The bacterial strain *Pseudomonas aeruginosa PAVIJ* was isolated from the petrochemical contaminated site among various other organisms and identified through biochemical tests accordingly to the standard procedure described by MacFaddin [9]. The strain was maintained on nutrient agar slants at 4^{o}C and sub-cultured continuously.

Extraction of biosurfactant from P. aeruginosa PAVIJ

A loop full of culture was transferred to 250ml Erlenmeyer flask containing 50 ml of mineral salt medium (MSM) containing 2% glucose as the sole carbon and energy source. Different concentrations of various carbon sources like Palm oil, Castor oil, Coconut oil and Honge oil in the range of 0.5-2.0% was added. The culture flasks were incubated at 30^{o}C for five days in a shaking incubator at 150 rpm. The cells were removed from the culture broth by centrifugation at 12,000 rpm for 15 minutes at 4^{o}C. The supernatant was filtered with 0.45µm Millipore filter paper and acidified to pH 3 with 2 N HCl and kept in freezer at 4^{o}C overnight. The biosurfactant was extracted by ethyl acetate on mild shaking at RT overnight. The solvent was evaporated and the oily residue was dissolved in 1ml of methanol and used for further screening.

Preliminary detection of biosurfactant by methylene blue complexation method

The presence of biosurfactant was recorded by the appearance of sky blue color in the bottom of the tube. The method relies on measuring the absorbance (at 638 nm) of the biosurfactant-methylene blue complex that partitions into the chloroform phase [10].

Estimation of biosurfactants by orcinol method

The positive sample for methylene blue complexation was analyzed for rhamnose concentration by the orcinol method according to Koch [11]. A standard curve was drawn up from L-rhamnose (100 µg/ml with a suitable blank at 421 nm and the concentrations of biosurfactants were expressed as rhamnose equivalents).

Purification of biosurfactants from P. aeruginosa PAVIJ by Silica G-60 column

The silica G60 column was regenerated with three volumes of 100% methanol for standardization. The ethyl acetate crude extract from *P. aeruginosa PAVIJ* treated with four different carbon sources was dissolved in 1ml of 100% chloroform. The chloroform was run through the column and allowed to equilibrate for five minutes. Equal volumes of chloroform : methanol in the ratio 9:1 was added to the column to remove the impurities. The gradient of methanol was made from 60% to 100% to completely remove the impurities and finally the biosurfactant was eluted with ethyl acetate and methanol in the ratio 7:3.

Screening methods

The purified extract were used for further screening to confirm for their biosurfactant production by performing different tests like, surface tension, E*24* (emulsification method), BATH assay, drop collapse, CTAB reduction and blood haemolysis [12-17]. The results were recorded and their mean values were tabulated.

Thin layer chromatography

A small quantity of column purified sample from *P. aeruginosa PAVIJ* treated with four different carbon sources (palm oil, castor oil, coconut oil and honge oil) was dissolved

in 20% methanolic chloroform and applied onto a TLC plate of 25-50µl (silica gel 60) at a point of origin near the bottom of the plate with standard rhamnolipids alongside for comparison. The plate was developed with the solvent system, chloroform: methanol: acetic acid in the ratio 65:15:2 (v/v/v) [18] and air dried.

The mono rhamnolipids and dirhamnolipids were detected by combining two methods to ensure the perfect rhamnolipids by their relative *Rf* values. The first method is spraying with p-anisaldehyde [anisaldehyde: sulphuric acid: glacial acetic acid 0.5:1:50] [19, 20] to spot carbohydrates and the second method is by spraying the plates initially with 1% α-naphtol solution and later by sulphuric acid: ethanol in the ratio 1:1 to spot fatty acids. The plates were heated in an oven at a temperature of 120-150^{o}C for 15-20mins for development of the color. Later the plates were observed for the colored spots, and their *Rf* was calculated.

HPLC analysis

The purified sample of biosurfactant from *P. aeruginosa PAVIJ* was taken and dried completely to remove ethyl acetate, the solvent used during purification. The dried samples were dissolved in 100% acetonitrile containing 2-bromoaceto-phenone and triethylamine in the molar ratio of 1:4:2 (biosurfactant:2-bromoacetophenone:triethylamine). The dissolved samples was kept in sonicator for 1h at 60^{o}C and filtered through 0.22mm Millipore filter to remove particulate material. 20µl of purified sample was injected and compared to the appropriate standard.

The HPLC was performed on an Agilent 1100 Series HPLC equipped a hydrophobic BDS HYPERSIL C-18 column of 250mm length, 4.6 mm internal diameter with 5µm particle size. Isocratic separation was done with water and acetonitrile as solvent system in the ratio 70:30 in the mobile phase. The chromatography was performed at ambient temperature with a flow rate of 0.8ml/min and injection volume of 20µl. The chromatography was monitored with an HPLC/UV detector at 272nm. Pure rhamnolipids Rha C10-C10 and Rha-Rha C10-C10 were used for calibration along with the testing samples.

Antimicrobial activity analysis

Determination of antimicrobial activity was performed by disc diffusion method. The standard disk diffusion method [21] was conducted by growing an even lawn of target organism on hard agar and placing disks impregnated with antimicrobial solution on the surface. The HPLC purified samples from all the four carbon sources treated samples from *P. aeruginosa PAVIJ* at the concentration of 10µg/ml were used for the analysis of antimicrobial activity. Plates of Mueller Hinton agar was evenly streaked in three different directions with a sterile cotton swab dipped in four different bacterial solutions of *Klebsiella pneumonieae, Escherichia coli, Staphylococcus aureus* and *Proteus* species (Obtained from clinical samples). Sterile round filter paper disks (5mm diameter) were dipped in the filtered extracts of *P. aeruginosa PAVIJ (*control dipped in sterile saline) and placed evenly on the agar surface with a pair of sterile forceps. The plates were incubated at 37^{o}C. The width of the inhibition zones including the disk diameter, were measured to the nearest whole millimeter after 48h using zonal scale.

Statistical analysis

Statistical analysis was performed by using SPSS 11.5 Windows software. The difference in mean biosurfactant production between different concentrations from different carbon sources were analyzed by applying two way ANOVA. The variations between different carbon sources in *Pseudomonas aeruginosa PAVIJ* for all the five screening tests were analyzed statistically with multiple ANOVA.

RESULTS AND DISCUSSION

After morphological and microscopic observations *Pseudomonas aeruginosa PAVIJ* was identified from petrochemical soil waste based on the colony characteristics and biochemical tests. The biosurfactant producing organisms can be isolated from the oil spilled soils [22]. This organism was selected for enhanced production of biosurfactants by treating with four different concentrations from 0.5 – 2.0% with various carbon sources (palm oil, castor oil, coconut oil and honge oil). The amount of biosurfactant produced was calculated according to the standard graph formed by standard rhamnose at the concentration of 25-100µg/ml and expressed as rhamnose equivalents (RE). Among the different concentrations of carbon sources used, 2% addition enhanced more of biosurfactant production in all the four types of oils used as shown in Figure 1.

It was also observed that the enhancement of biosurfactant was more with palm oil treatment compared to the other three oils. Castor oil also proved to be a good enhancer for biosurfactant production, but comparably lesser than palm oil. Coconut oil and honge oil showed poor response with little increase in biosurfactant production when compared with the untreated sample. *Pseudomonas aeruginosa PAVIJ* was considered as a very potent organism for the induction of biosurfactant production and hence its extract was selected for further chromatographic analysis.

The strains of *Pseudomonas* sp. selected for enhanced production by palm oil and castor oil treatments produced mainly rhamnolipid biosurfactants. Basically, biosurfactants are frequently classified according to the organism which produces them. Rhamnolipids are extracted from *Pseudomonas* sp, the lipopeptides, surfactin and iturin from *Bacillus,* glycoside and rhamnolipids from *Streptococcus* sp. [23] and *Micrococcus* sp.[24] *Rhizobium* sp. is also found to produce rhamnolipid type of biosurfactant [25]. It indicates in few of the organisms the three *lactobacillus acidophilus* strains inhibit the integrity of biofilm formation. Biosurfactant have a wide variety of structures which means that no standard method can be applied to determine the concentration of all biosurfactants directly. In this study, orcinol assay-a colorimetric method was used for direct biosurfactant quantification [26] .Quantification of biosurfactants was done by using standard graph obtained from L-rhamnose (0-100 µg/ml) and the amount of biosurfactants was expressed as rhamnose equivalents (RE mg/ml) [27].

Qualitatively different tests were performed for *P.aeruginosa PAVIJ* to confirm for biosurfactant production, surface tension, drop collapse, emulsification assay, BATH assay, CTAB reduction test and hemolytic activity were used. In all these tests *Pseudomonas* sp exhibited good activity and this is encompassed by their rhamnolipid production in higher

concentration [28,29]. The anionic surfactant was determined on CTAB medium in which colonies were surrounded by the blue dark color halos for palm oil and castor oil extracts as shown in Figure 2.

The variations between palm oil, castor oil, coconut oil and honge oil in *Pseudomonas aeruginosa PAVIJ* for all the five screening tests were analyzed statistically with multiple ANOVA as shown in Table 1.The statistical hypothesis was accepted for the differences in the screening methods showing variations at 0.001% level of significance.

Purified biosurfactant was eluted by normal phase column chromatography which was carried out for the extracts obtained from four different carbon sources. This normal phase chromatography with silica gel and ethyl acetate is a standard method with more than 90% purification for biosurfactants [30-32]. Reversed-phase chromatography on a C18 column with a gradient of acetonitrile and water is another option [33]. Ion exchange chromatography with DEAE sepharose can be applied as well [31,34]. The biosurfactants are eluted from the ion exchange resin by increasing salt concentrations. Adsorption chromatography with hydrophobic Amberlite XAD-2 or XAD-16 polystyrene resin can be used for first biosurfactant enrichment from the culture supernatant [34,35].

The Thin layer chromatography for *P. aeruginosa PAVIJ* extracts obtained from different carbon source was performed according to Syldatk [36]. The ethyl acetate extracts from *Pseudomonas* supplemented with palm oil and castor oil produced two prominent spots with *Rf* value of 0.7 and 0.3 whereas castor oil and honge oil treated extract produced one spot with *Rf* value of 0.7. The observation of these glycolipids was done by using two different detecting reagents like p-anisaldehyde which stains carbohydrates blue (Figure 3) and α-naphtol with 50% ethanolic sulphuric acid which stains fatty acids yellow (Figure 4). The spots were confirmed with the rhamnolipid standards corresponding to Rha C10-C10 and Rha Rha C10-C10.

The two rhamnolipids obtained after chromatography reveals Rha C10-C10 and Rha-Rha C10-C10 when compared with the standard. The other spots might be the rhamnolipids for which the comparison could not be done due to the unavailability of corresponding standards or the impurities like triglycerides and few fatty acids. The *Rf* values obtained by previous study was helpful in detecting the particular rhamnolipid, where in the lesser *Rf* values corresponds to dirhamnolipid, Rha-Rha C10-C10 and higher *Rf* values to mono rhamnolipid, Rha C10-C10 [36]. The presence of carbohydrates induces less hydrophobicity to the compound and hence the dirhamnolipid had lesser *Rf* values when compared to the monorhamnolipid [31].The different detection system is one of the additional applied aspect of rhamnolipids because the availability of these is easier and do act as an important confirmatory agents during detection [37]. There are numerous detection systems available, but p-anisaldehyde detection combined with α-naphtol detection proves to be the best for rhamnolipid detection as the blue-green colour separates the rhamnolipids from other fatty acids and yellow color of α- naphtol confirms the fatty acids [36, 18, 32].

The main rhamnolipids of *P. aeruginosa*, namely, Rha C10-C10 and Rha-Rha C10-C10 was quantified by HPLC method where no standard for the other rhamnolipids was available. The retention times of standard and extracted samples matched each other and

hence quantification was made according to the area of the peak formation and expressed as mg/100ml of the inoculated media as represented in Table 2 and Figure 5.

The isolates of *P. aeruginosa PAVIJ* strain from all the four different carbon sources showed difference in their rhamnolipid type and content. Rha C10-C10 content was less when compared to Rha-Rha C10-C10. The Rha C10-C10 was highest in castor oil (276mg/100ml), followed by coconut oil (181mg/100 ml), palm oil (170mg/100 ml) and honge oil (0.77mg/100 ml). The Rha Rha C10-C10 content was more in palm oil isolate of 979mg/100 ml, 60.25mg/100 ml in castor oil isolate, 47.2 mg/100 ml in coconut oil and 85.8mg/100 ml in honge oil isolate as shown in Plate-1. Column chromatography with gradient elution according to Andra [38] is the feasible method for the purification of biosurfactants from *P. aeruginosa*. This technique gave the optimized result with reduction in organic solvent consumption.

HPLC method is one of the suitable methods developed to quantify rhamnolipids in a bacterial biosurfactant mixture. In the present study mainly Rha C10-C10 and Rha-Rha C10-C10 are the two rhamnolipids produced from the *P aeruginosa PAVIJ* strain with higher concentration of Rha Rha C10-C10. Among the four carbon treatments palm oil treatment proved best with high production of both the rhamnolipids. Castor oil treated sample also showed good response in production. The coconut and honge oil treated samples showed only one rhamnolipid, Rha C10-C10 with very less concentration. These results correlated with other literatures, where L-Rhamnosyl-L-rhamnosyl-b-hydroxydecanoyl-b-hydroxydecanoate and L-rhamnosyl-b-hydroxydecanoyl-b-hydroxydecanoate, referred to as rhamnolipid 1 and 2, respectively (Rha C10-C10 and Rha-Rha C10-C10) are the principal glycolipids produced by *P. aeruginosa* [18]. The study done by earlier researchers shows that with the presence of a suitable standard rhamnolipid the quantification of a particular rhamnolipid within the mixture of the extracted supernatant can be analyzed easily. Similarly, the biosurfactant mixtures containing mainly 3-3'-(L-rhamnopyranosyl-oxy)decanoyloxydecanoic acid (Rha C10-C10), 3-3'-(2'-O-alpha-L-rhamnopyranosyl-oxy)decanoyloxydecanoic acid (Rha Rha C10-C10), 3-3'-(2'-O-alpha-L-rhamnopyranosyl-oxy)decanoyloxydodecanoic acid (Rha Rha C10-C12), and a dehydrogenated variety of the latter, 3-3'-(2'-O-alpha-L-rhamnopyranosyl-oxy)decanoyloxydodecenoic acid (Rha Rha C10-C12-H2), were isolated from *Pseudomonas aeruginosa* UG2 cultures grown on corn oil as sole carbon and analyzed with the standards by HPLC-UV detection method [39]. Another study was done on production of rhamnolipids by *Pseudomoas* sp. DSM 2874, with rape seed oil as a carbon source. The analysis was done by HPLC-UV detection method and the product obtained from rapeseed oil was found to be a mixture of up to four glycolipids (rhamnolipid 1 - 4), L-(+)rhamnose and (*R*, *R*)-3-(3-hydroxydecanoyloxy) decanoic acid [40].

Antimicrobial activity was determined for the purified extracts of *P. aeruginosa PAVIJ* by measuring the zone of inhibition. A positive control amphicillin at the concentration of 100µg/ml (tested sensitive in the laboratory, Table - 3) and a negative control saline was used together with all the four carbon treated samples of *P. aeruginosa PAVIJ* in this study. The inhibitory concentration for rhamnolipids at 10µg/ml was used to determine antimicrobial activity and zonal scale was used to measure the zone of inhibition.

Table- 1: Mean values in combination of *PAVIJ* and carbon sources for screening of biosurfactants

Bacteria	Carbon Source	Mean ±S.D. ±S.E.				
		Drop collapse test	Bath assay	Surface tension	Emulsification index	Blood hemolysis
Pseudomonas aeruginosa *PAVIJ*	Palm oil	0.73 ± 0.02± 0.01	120.70 ±1.06 ± 0.33	30.26 ± 0.53 ± 0.17	46.37 ± 0.59 ± 0.19	4.10 ± 0.18 ± 0.06
	Castor oil	0.51 ± 0.01 ± 0.00	144. 91 ±2.35 ± 0.74	34.12 ± 0.01 ± 0.00	42.10 ± 0.04 ± 0.01	3.77 ± 0.04 ± 0.01
	Coconut oil	0.53 ± 0.02 ± 0.01	50.80 ± 1.03 ± 0.33	49.77 ± 0.28 ± 0.09	41.70 ± 0.31 ± 0.10	3.73 ± 0.29 ± 0.09
	Honge oil	0.41 ± 0.01 ± 0.00	73.70 ± 1.77 ± 0.56	52.43 ± 0.10 ± 0.03	35.92 ± 0.02 ± 0.01	2.41 ± 0.17 ± 0.05
	Untreated	0.32 ± 0.02 ± 0.01	47.30 ± 2.06 ± 0.65	62.49 ± 0.15 ± 0.05	22.18 ± 0.07 ± 0.02	0.00 ± 0.00 ± 0.00

Table-2: Amount of biosurfactants produced from PAVIJ strain as analyzed by HPLC

Serial No.	*PAVIJ* extracts	*P. aeruginosa* mg/100ml	
		RL_1	RL_2
1	Palm oil	170±2.23	979 ± 2.99
2	Castrol oil	276±2.44	60.25 ± 1.77
3	Coconut oil	181±2.25	47.2 ± 1.67
4	Honge oil	0.77±0.11	85.8 ± 1.93
5	Untreated	0.32±0.49	---

Table-3: Profile of the pathogenic microorganisms taken for antimicrobial activity

Serial No.	Bacterial strains*	Isolation site	Resistant pattern ¥
1	*Klebsiella pneumoniae*	Ascitic fluid	Resistant to ceftriaxone, ciprofloxacin. Ofloxacin, levofloxacin, norfloxacin, piperacillin,s treptomycin and penicillin
2	*Proteus sp.*	Urine sample	Resistant to ciprofloxin, norfloxacin
3	*E.coli*	Stool sample	Resistant to penicillin, treptomycin, ciprofloxin, norfloxacin,ofloxacin, ceftriaxone, ceftazidine
4	*Staphylococcus aureus*	Nasal swab	Resistant to penicillin, methicillin and streptomycin

*Strains collected from Clumax Diagnostic Centre, Bangalore, India.

¥ As indicated by the Hospital records.

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Table 4: In-vitro testing of antimicrobial activity of biosurfactant fractions of PAVIJ against bacterial strains by agar disc diffusion method.

PAVIJ extracts	Antimicrobial zone diameter (mm)			
	K. pneumoniae	*Proteus sp.*	E. coli	*S. aureus*
Palm oil	<10	19	14	--
Castrol oil	14	18	14.5	--
Coconut oil	5	--	--	--
Honge oil	6	--	12.5	--
Control	--	--	--	--
Pencillin (100µg/ml)	18	14	14	12

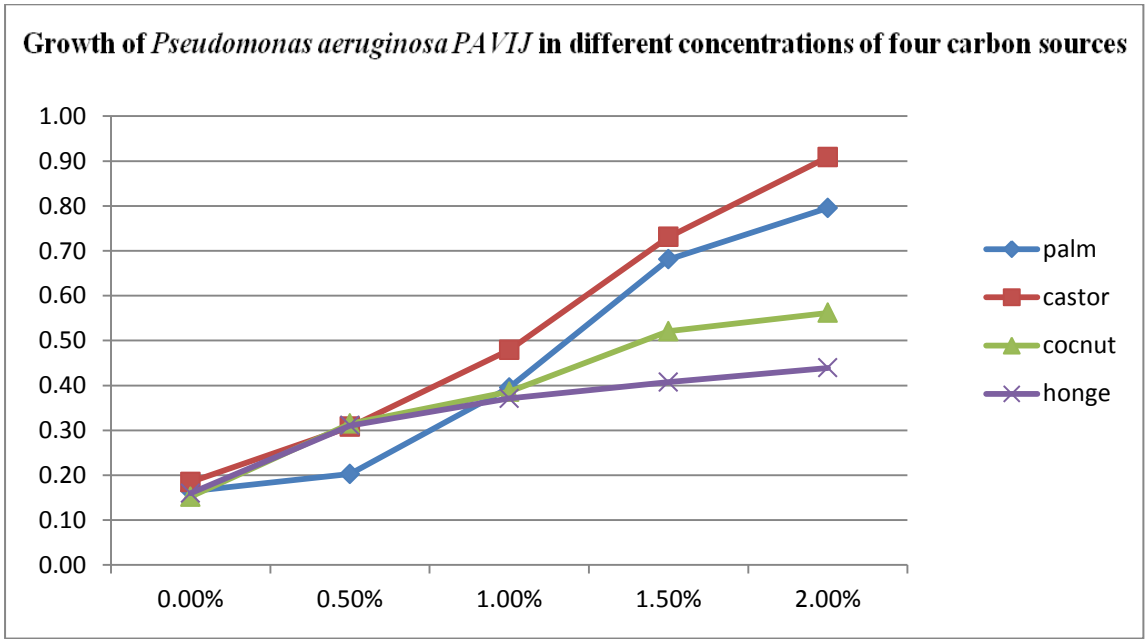

Fig .1 Growth of *PAVIJ* in different concentrations of carbon sources

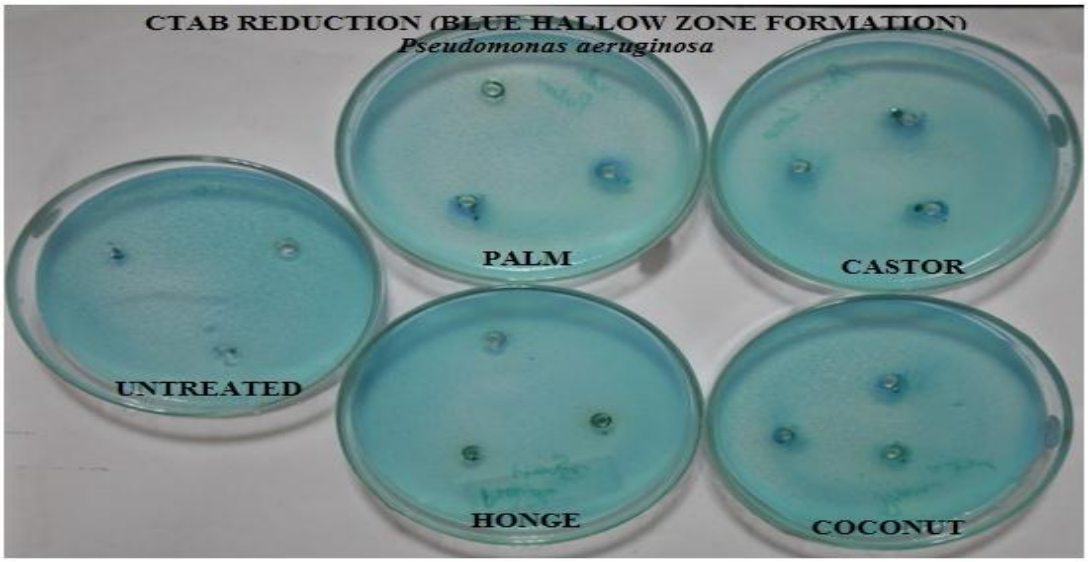

Fig.2. CTAB agar test of PAVIJ

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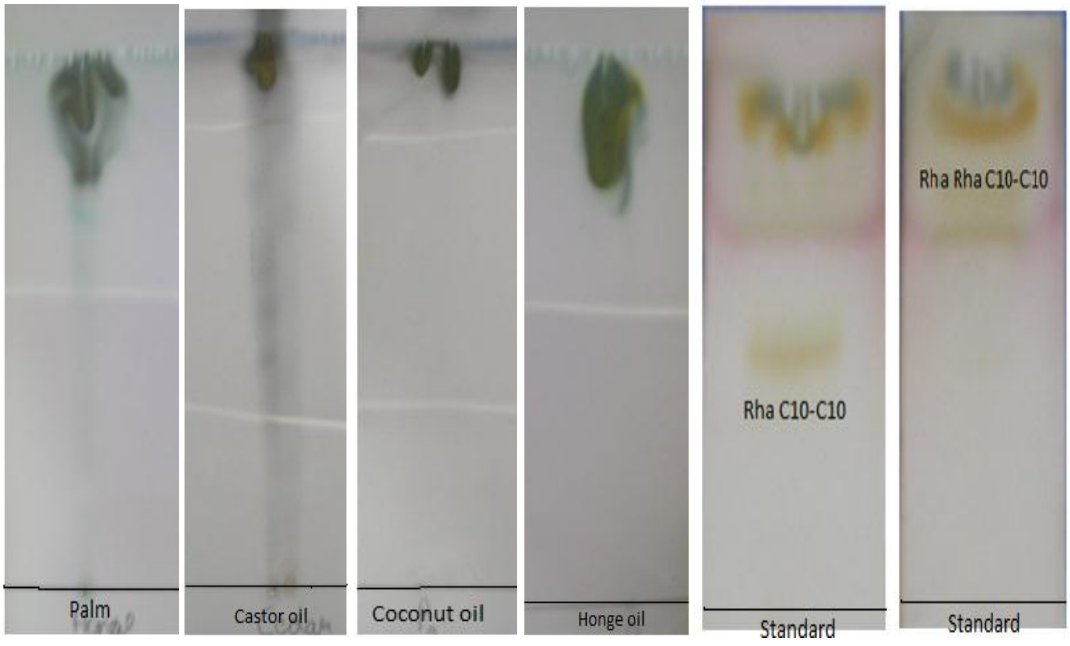

Fig-3: TLC plates - blue-green spots of rhamnolipids when stained with p-anisaldehyde

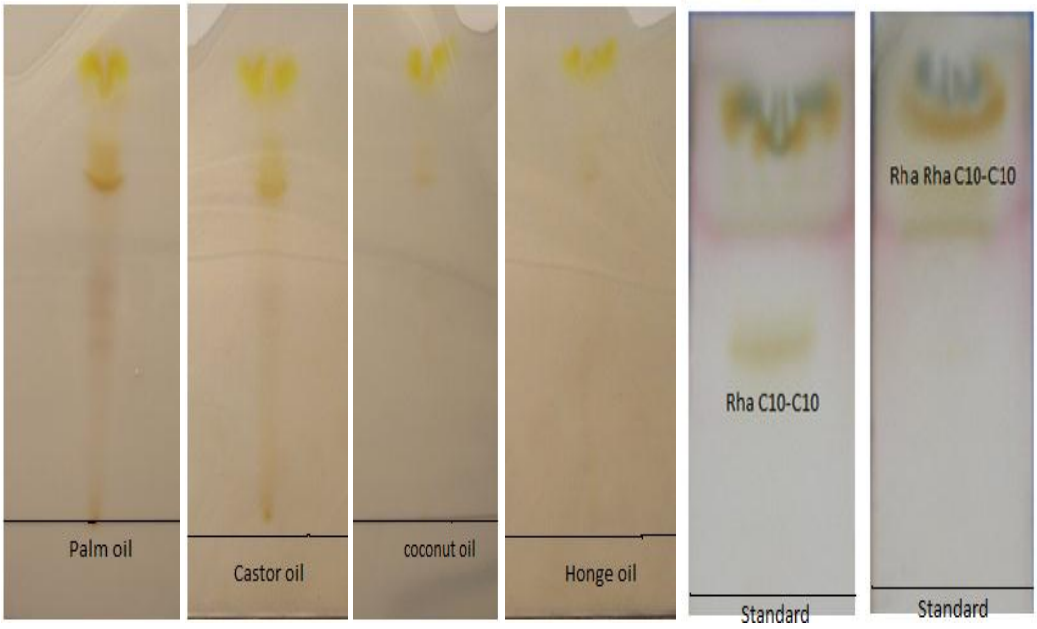

Fig 4- TLC plates - yellow spots of rhamnolipids when stained with α-naphtol and con., H_2SO_4

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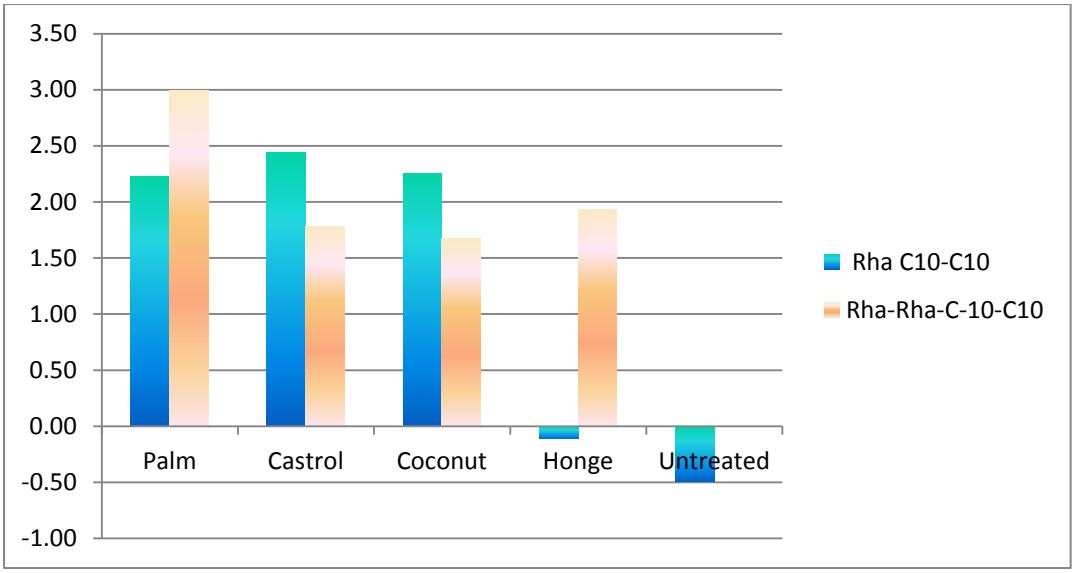

Fig 5- Amount of rhamnolipids produced from *PAVIJ* by HPLC expressed as SD ± mg/100ml

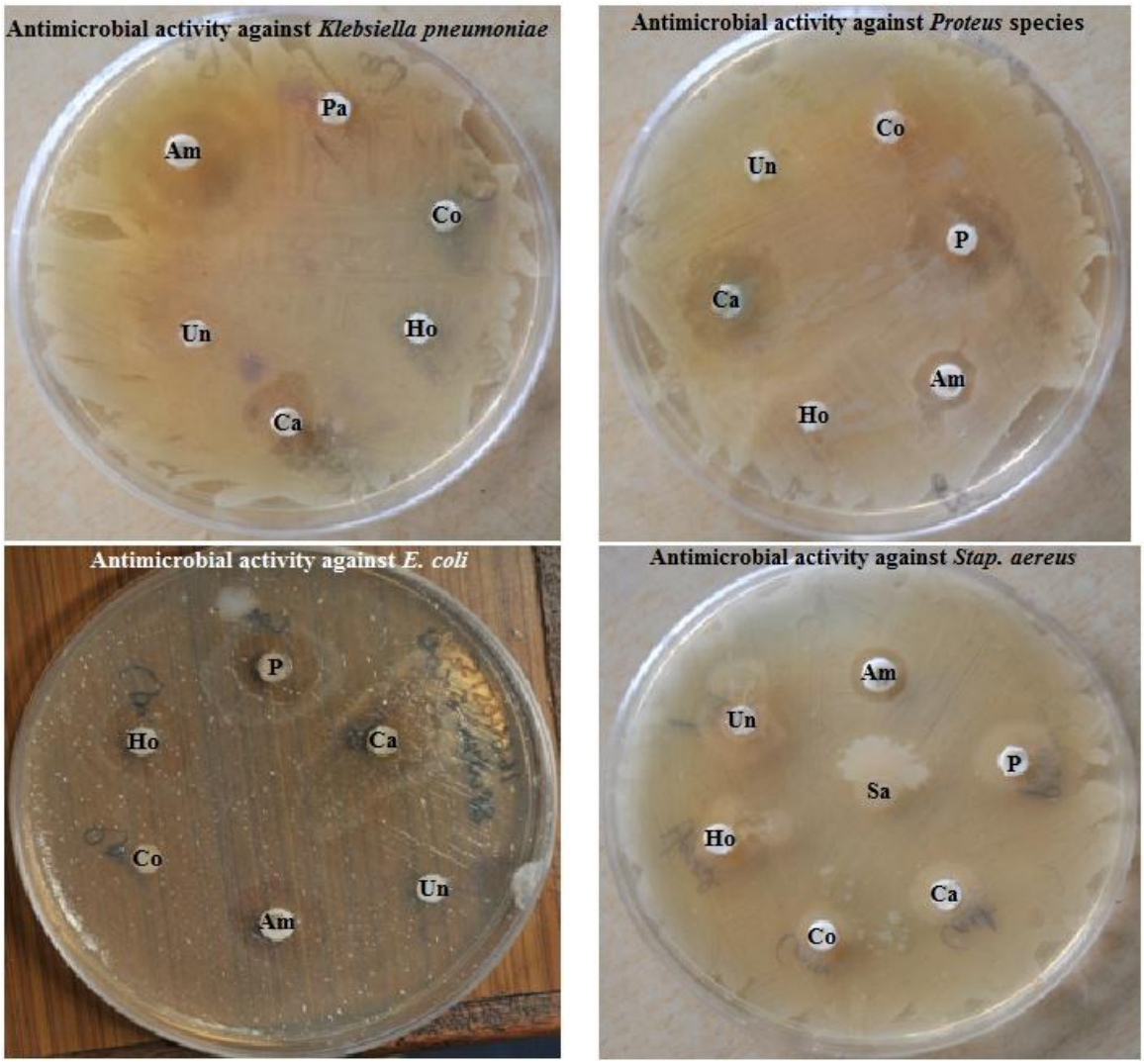

Fig-6: Antimicrobial activity *of PAVIJ* isolate against different organisms

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PLATE-1

HPLC chromatogram of rhamnolipids from *PAVIJ* for different carbon sources

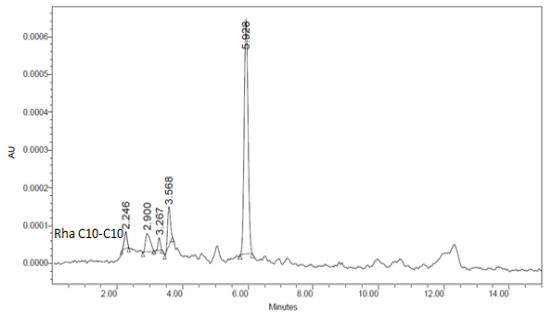

Fig- A: Untreated sample of *PAVIJ* with Rha C10-C10

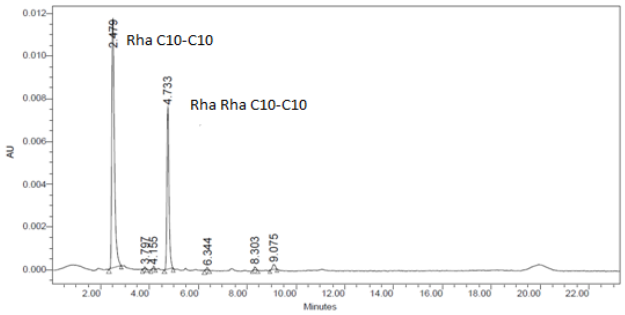

Fig-B: Standards-Rha C10-C10 and Rha Rha C10-C10

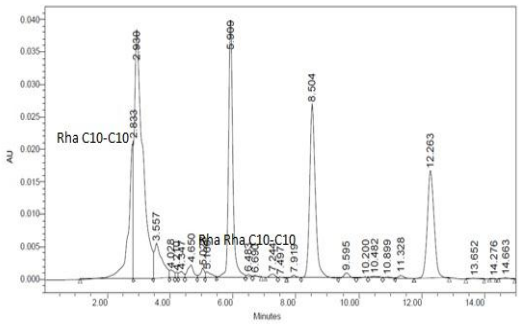

Fig-C: Palm oil isolates with Rha C10-C10 and Rha Rha C10-C10

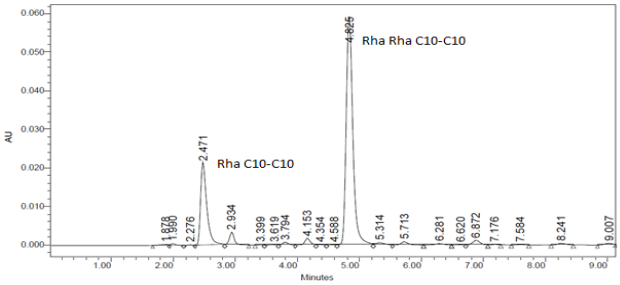

Fig-D: Castor oil isolate with Rha C10-C10 and Rha Rha C10-C10

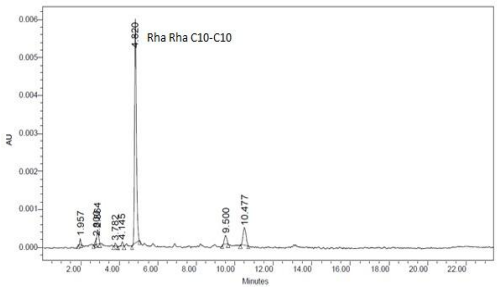

Fig-E: Coconut oil isolate with Rha C10-C10 and Rha Rha C10-C10

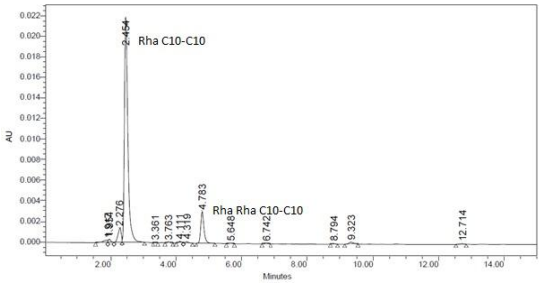

Fig-F : Honge oil isolate with Rha C10-C10 & Rha Rha C10-C10

The biosurfactants Rha C10-C10, Rha-Rha C10-C10 from *P. aeruginosa PAVIJ* strain showed antimicrobial activity against almost all the micro-organisms tested (*Klebsiella pneumonieae, Escherichia coli* and *Proteus* species) except against *Staphylococcus aereus* (Table. 4 and Figure 6) .

For *K. pneumonieae* rhamnolipids from Castrol treated sample showed higher antimicrobial activity with 14mm zone of inhibition, whereas palm, coconut and honge oil treated samples showed very less activity with 10mm, 5mm and 6mm zone of inhibition. For *Proteus* species palm and castor oil treated samples showed very high inhibition of 19mm and 18mm diameter which is higher than the positive control amphicillin was seen whereas the coconut and honge isolates did not show any inhibitory activity. For *Escherichia coli* palm, castor and honge oil isolates showed similar effects with almost same zone of inhibition around 13-14mm diameter and coconut isolate showed no response.

The focus of the study presented here is the use of biosurfactants as antimicrobial agents against organisms like *Klebsiella pneumoniae, Proteus sp.* and *E. coli*. In order to illustrate the enormous potential of biosurfactants as biomedical compounds, the antimicrobial activity of rhamnolipids was analyzed and briefly discussed.

The rhamnolipids produced from *Pseudomonas aeruginosa PAVIJ* strain showed very good antimicrobial activity against all the tested organisms. Their detergent nature makes them very potent antimicrobial agents. Rhamnolipids have been shown to display antibacterial activities against plant and human pathogenic bacteria and are known to be active against the Gram-negative bacteria *P. aeruginosa*, *Enterobacter aerogenes*, *Serratia marcescens* and *Klebsiella pneumonia*, as well as against Gram-positive *Micrococcus* sp., *Streptococcus* sp., *Staphylococcus* sp. and *Bacillus* species [7,41] observed a loss of lipopolysaccharides (LPS) in *P. aeruginosa* strains treated with rhamnolipids at low concentrations and this resulted in increased cell surface hydrophobicity. Recently, [42] showed that rhamnolipids from *Pseudomonas* PS-17 interact with *P. aeruginosa* causing a reduction in LPS content and changes in the outer membrane proteins of the bacteria. These changes had a direct impact on bacterial cell surface morphology concluding rhamnolipids from *Pseudomonas* species have a potential application in the field of biomedicine against pathogenic bacteria.

CONCLUSION

In this study, we were successful in obtaining a very potent organism namely *P. aeruginosa PAVIJ s*train from a petrochemical contaminated soil. The organism was grown on four different carbon sources of different concentrations to enhance the biosurfactant production.

Screening was carried out with standard procedures, with series of experiments conducted for its confirmation. The analyses showed the presence of mixture of both mono and dirhamnolipids Rha C10-C10 and Rha-Rha C10-C10 from 2% palm and castor oil treated samples. With purified samples of rhamnolipids Rha C10-C10 and Rha-Rha C10-C10 the characterization was done and confirmed. They have a potential market as novel antimicrobial agents. Mono and dirhamnolipids from *P. aeruginosa* PAVIJ strain at a concentration of 10µg/ml led to a zone of inhibition of 18-19mm for *Proteus* species. This concentration is 350% more than the standard antibiotic amphicillin used at the concentration of 100µg/ml with the inhibition zone of 14mm. The result from the study reports that even from the cheapest carbon sources like palm and castor oil at a very lesser concentration of 2% a good concentration of biosurfactants can be produced. This has opened up a practically significant and commercially viable biotechnological approach to produce varieties of biosurfactants having huge industrial application. The production yield must be maximized since the activity was shown to be directly related to concentration. Higher yields also improve the economics of the process, which is important for commercialization of the product.

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