iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 6 **Issue:** IV **Month of publication:** April 2018

DOI: http://doi.org/10.22214/ijraset.2018.4379

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
Volume 6 Issue IV, April 2018- Available at www.ijraset.com

Comparative Study of Biosurfactant Producing Organisms Isolated from Pesticide and Oil Contaminated Soil

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Abstract: ***Biosurfactants are diverse groups of surface active molecules synthesized by microorganisms which help in stabilizing emulsion, enhancing foaming and reducing surface tension. In this study, six soil samples were collected from pesticide and oil contaminated sites. Each sample was enriched in mineral salt medium then serially diluted and pour plated in the nutrient agar plates. A total of 32 morphologically distinct isolates were isolated in pure form from soil samples which were screened for the purpose of biosurfactant production. To confirm and compare the ability of isolates in biosurfactant production from different sources, different screening methods including foaming, emulsification index E24, drop collapse method and oil spreading test were assessed. Curd whey was used as a low cost growth medium for biosurfactant producing organisms. Kocuria rosea from pesticide contaminated soil and Micrococcus luteus/ lylae from oil contaminated soil sample were potent biosurfactant producers which were furthermore identified. Kocuria rosea showed highest emulsification index and prominent zone for oil spreading assay as compared to Micrococcus luteus/ lylae which suggested that biosurfactant producers isolated from pesticide contaminated soil sample have higher potency than biosurfactant producers isolated from oil contaminated soil sample.***
Keywords: Biosurfactant, Kocuria rosea, Micrococcus luteus/lylae, emulsification index, oil spreading, drop collapse test.

I. INTRODUCTION

Now a days use of oils and petroleum hydrocarbons is increased as main energy sources that may lead to leakage of these products while transportation, leading to soil and water pollution; which is not beneficial for human beings, microbes, plants and animals thus the demand for biosurfactant is rapidly increasing (Kosaric, 2001). Biosurfactants have been tested in environmental solution such as bioremediation and dispersion of pesticides, enhanced oil recovery and transfer of crude oil through pipelines, and it is thought to be the potential candidate to replace synthetic surfactants in the future, especially in the food and health care industries, industrial cleaning of oil coated surfaces and in agricultural chemicals (Banat *et al*., 2000, Makkar and Cameotra 2002, Karanth *et al*., 1999, Mukherjee *et al*., 2006). They have gained a remarkable importance in the fields of environmental bioremediation, pharmaceuticals and food processing (Muthusamy *et al*., 2008).

They are mostly used because of their environment friendly nature, easy degradability, low toxicity and capable of production from cheaper substrates (Meybodi, et al., 2013).

Biosurfactants are amphiphilic biological compounds produced extracellularly or as part of the cell membranes by a variety of bacteria, yeast and filamentous fungi (Batool *et al*., 2017). They can reduce surface, interfacial tension (Rosenberg and Ron, 1999) thus can potentially be used in enhancing oil recovery, and bio emulsification processes (Maier *et al.*, 2003). Depending upon polarities of biosurfactant, they make interfaces with various solution such as oil and water which results in lowering the interfacial tension (ElSheshtawy, *et al*., 2015).

Petroleum, oil related industry and pesticide industry found to have a greater potential to produce biosurfactant-producing organisms. Surfactants may improve the bioavailability of hydrophobic compounds to their potential degraders (Noordman and Janssen 2002). In the present study, the biosurfactant producing microorganisms were isolated, screened, characterized for the biosurfactant production.

The present study also focused on the isolation of efficient biosurfactant producers at optimized conditions in low cost growth medium i.e. curd whey to reduce the production cost of biosurfactant. After screening tests, efficiency of the biosurfactant producers isolated from pesticide and oil contaminated soil samples was compared.

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II. MATERIALS AND METHODS

A. *Sample area and sampling*

Soil samples were collected from Yawalkar pesticide industry, Kamthi Road, Nagpur and oil contaminated soil samples from garage, near Khapri, Nagpur at 3 different locations each. The samples were taken in sterile polythene bag and preserved in refrigerator at 4^0C for further use which was analyzed for the isolation of biosurfactant producing bacteria.

B. *Enrichment and Isolation of biosurfactant producing microbes*

1 gm of collected soil was inoculated in 100 ml sterile Mineral salt medium broth (MSM) with 2% (v/v) liquid paraffin and incubated at 37^0C for 7 days at 180 rpm (Patil, *et al*., 2012). The composition of the mineral salt medium was modified in our laboratory as follows: $NaNO_3$ -3 gm; KH_2PO_4 -1.5 gm; Na_2PO_4 -1.5 gm; $MgSO_4.7H_2O$ -2 gm; $CaCl_2.2H_2O$ -0.005 gm; $FeSO_4$ -0.001 gm; $ZnSO_47H_2O$ -70 µg; $CuSO_4.5H_2O$ –50 µg; H_3BO_3 -10 µg; MoO_3 -10 µg per liter; pH 7±0.2. After 7 days 1 ml inoculums were transferred to the same medium and incubated at 37^0C for another 7 days. The process was repeated for three times, 1 ml inoculum from the third enrichment culture was appropriately serially diluted, and 100 µl aliquots from the last two dilutions were streaked on nutrient agar plate. From this, organisms were isolated.

C. *Storage of bacterial isolates*

The selected bacterial isolates were stored in nutrient agar slants for further identification. These cultures were maintained at 37^0C on nutrient agar slant.

D. *Screening of biosurfactant producing organisms*

The isolated pure cultures were transferred separately to culture tubes containing 30 ml mineral salt medium with 2% (v/v) liquid paraffin and incubated in orbital shaker for 5 days at 180 rpm at 37°C. After the incubation period the tubes were vortexed for 2 min to record the foaming and
turbidity of the growth medium. Medium without inoculum served as control.

E. *Culture supernatant*

After the incubation period of 5 days, mineral salt medium containing biosurfactant was centrifuged at 10,000 rpm for 10 min and supernatants of biosurfactant producing organisms were used for testing the biosurfactant production by following methods.

F. *Emulsification index E24*

Emulsification index of culture samples were determined by adding 2 ml of oil (vegetable oil, petrol, kerosene and diesel) to the same amount of culture supernatant, mixing with a vortex for 2 min, and allowed to stand for 24 hours. The emulsification index was calculated as percentage of height of emulsified layer (cm) divided by total height of the liquid column (cm) (Sarubbo, 2006).

G. *Oil Spreading Technique*

20µl of crude oil (Kerosene, Vegetable oil, Petrol and diesel oil) was added to the surface of 50 ml of distilled water in a glass petri dish (15 cm diameter) to form a thin oil layer and finally 10µl of culture supernatant of all the 40 isolates was added and compared to observe the clearance zone (Rodrigues, *et al*., 2006).

H. *Drop collapse method*

Method of Youssef, *et al.,* (2004) was used to test the presence of biosurfactant. A single drop of culture supernatants were placed on the solid surface coated with oil. After 1 min the shape of the drop was observed on the oil surface. Flat shaped drops were indicative of positive result for biosurfactant production whereas round drops showed negative results of biosurfactant production.

I. *Foaming test*

Isolated colonies were incubated separately in 100 ml flasks, each containing 25 ml processed and sterilized MSM. After the incubation period of 5 days at 37^0C the flasks were vortexed for 2 min to record the foaming of the growth medium.

J. *Stability of foam*

Isolated colonies were grown separately in 250 ml Erlenmeyer flasks, each containing 100 ml of mineral salt medium. The flasks were incubated at 37°C on a shaker incubator (180 rpm) for 5 days. Foam produced by hand shaking of the fermented culture broth for several minutes was observed for its stability for a period of two h (Abouseoud *et al*., 2007).

K. Curd whey as a low cost growth medium for biosurfactant producing organisms

The processing of curd whey was done by adjusting the pH 4.1 to 7 by using 5 N NaOH and heating for 2-3 times. After adjusting the pH, whey was cooled and centrifuged at 8000 rpm for 12 min to remove casein. Supernatant was adjusted to pH-7.0 again and 30 ml of processed curd whey was distributed in a big culture tube and sterilized at 121^0C for 20 minutes. Sterile whey was separately inoculated with 32 isolated colonies and incubated in orbital shaking incubator at 180 rpm for 96 hours (optimum biosurfactant production time). Medium without inoculum served as control. Fermented whey containing biosurfactant was centrifuged at 10,000 rpm for 10 min.

L. Screening for biosurfactant activity in curd whey as a growth medium and screening of an efficient biosurfactant producing organisms in curd whey

Screened biosurfactant producing microbial isolates in MSM medium were assessed qualitatively and quantitatively to screen for the efficient isolates in curd whey as growth medium. Efficiency of screened biosurfactant producing organisms grown in MSM medium was again checked by emulsification assay, oil spreading technique, stability of foam, drop-collapse test (as mentioned in 2.4) when curd whey was used as a growth medium.

M. Characterization and identification of biosurfactant producing organisms

According to MacFaddin (2000), the best two biosurfactant producing bacterial isolates, BS-4 and BS-25 each from pesticide and oil contaminated soil respectively were characterized by gram staining, morphological characteristics and biochemical reactions. Results were compared with Bergey's Manual of Systematic Bacteriology. BS-4 and BS-25 were further identified to species level using sequencing as *Kocuria rosea* and *Pseudomonas aeruginosa* from Yaazh Xenomics, Mumbai, India.

N. Extraction of biosurfactant in MSM and curd whey

Extraction was done by using acetone and then precipitation by HCI, overnight at 4°C. The product recovered was dried under vacuum and preserved. The culture broth was centrifuged (10000 g, 15 min) to remove the cells and thereafter sterilized with millipore membrane filter. The clear sterile supernatant served as the source of the crude biosurfactant. The biosurfactant was recovered from the cell free culture supernatant by cold acetone precipitation (Sarubbo, 2006).

III. RESULTS AND DISCUSSION

A. Isolation and screening of biosurfactant producing microbes

Pesticide contaminated soil samples from the pesticide industry and oil contaminated soil from garage were collected. The pH of the sample during sample collection was 7. The temperature of the soil sample during sample collection was 30^0C. From the nutrient agar, 32 bacterial cultures, BS-01-BS-32 (17 from pesticide contaminated soil and 15 from oil contaminated soil) being morphologically distinct isolates were isolated and tested for the efficient biosurfactant production by plate and dilution technique. Colonies were purified and stored at 37^0C on nutrient agar slant for further analysis. All the isolates showed good growth in mineral salt medium containing 2% (v/v) liquid light paraffin as the sole carbon source.

B. Curd whey as a growth medium

Processed curd whey had been used as a good and cheap substitute for the production of biosurfactant. All the 32 isolates showed good biosurfactant productivity when inoculated in processed curd whey. After centrifugation of the curd whey, supernatants of biosurfactant producing organisms were used for testing the biosurfactant screening tests.

C. Screening of biosurfactant producing bacteria in MSM medium and curd whey medium

Based on screening methods viz. emulsification index, oil spreading test, drop collapse method, foaming test and stability of foam, it was proved that all the 32 bacterial isolates showed positive results for biosurfactant production in both the growth medium (Table 1). Combination of foaming, emulsification index and oil spreading tests are commonly used to identify microbes as potential biosurfactant producers (Satpute, *et al*., 2008). All the 32 strains, BS1-BS32 were further screened for biosurfactant activities by emulsification index, drop collapse method, oil spreading technique, blood hemolytic test, stability of foam and, as reported by Satpute *et al*. (2008) that more than one screening methods should be included in the primary screening as to identify potential biosurfactant producers.

Aqueous solutions of both BS-4 and BS-25 biosurfactants showed good foaming stability. Total disappearance of the foam was detected after 2 hours. Stabilization of an oil and water emulsion is commonly used as a surface activity

indicator. The results of emulsification activity and clearance zone produced by oil spreading technique are shown in Table 1. All the hydrocarbons tested served as substrates for emulsification by the biosurfactant. Diesel and kerosene were the best substrates for both the strains while vegetable cooking oil was less good substrates for emulsification.

All the 32 strains, BS1-BS32 were further screened for biosurfactant activities by emulsification index, drop collapse method, oil spreading technique, blood hemolytic test, stability of foam and, as reported by Satpute *et al*. (2008) that more than one screening methods should be included in the primary screening as to identify potential biosurfactant producers.

The drop collapse test and oil displacement tests are indicative of the surface activities. Drop collapse test and oil displacement test were highly positive for BS4 and BS25 as compared to the other 30 cultures.

Table 1 Detection and comparison of biosurfactants producing isolates in MSM and curd whey medium by preliminary and complementary screening methods screening methods

Bacterial Isolates	Emulsification Index (E24%)		Drop collapse test		Foaming test		Foam Stability Course (min)	
Bacterial Isolates of pesticide contaminated soil	MSM	Curd whey	MSM	Curd whey	MSM	Curd whey	MSM	Curd whey
BS-1	53	57	+	+	+	+	110	123
BS-2	42	51	+	+	+	+	125	129
BS-3	60	65	+	+	+	+	126	132
BS-4	70	72	+	+	+	+	128	139
BS-5	65	70	+	+	+	+	82	95
BS-6	41.5	50	+	+	+	+	94	100
BS-7	58.7	65	+	+	+	+	100	112
BS-8	61	61.3	+	+	+	+	54	67
BS-9	49.5	59.2	+	+	+	+	115	121
BS-10	61.5	63.2	+	+	+	+	100	120
BS-11	52.9	55	+	+	+	+	104	115
BS-12	58	61	+	+	+	+	120	129
BS-13	51	60.6	+	+	+	+	66	79
BS-14	65	68.85	+	+	+	+	125	130
BS-15	70.7	70	+	+	+	+	120	128
BS-16	52	58.6	+	+	+	+	121	122
BS-17	60	65	+	+	+	+	98	110
Bacterial isolates of oil contaminated soil								
BS-18	40	48	+	+	+	+	72	76
BS-19	44.5	51.5	+	+	+	+	88	92
BS-20	51.7	56.5	+	+	+	+	107	110
BS-21	60	62	+	+	+	+	100	106
BS-22	52	58	+	+	+	+	110	120
BS-23	51.6	54.6	+	+	+	+	109	115
BS-24	49	54	+	+	+	+	51	53
BS-25	64	65.55	+	+	+	+	125	137
BS-26	44.5	52.5	+	+	+	+	120	130
BS-27	36.8	49.8	+	+	+	+	110	121
BS- 28	41.2	42.2	+	+	+	+	98	100
BS-29	42	52	+	+	+	+	56	65
BS-30	60	63	+	+	+	+	58	59
BS-31	38	43	+	+	+	+	110	120
BS-32	32	43	+	+	+	+	101	110

D. Findings are the mean values of the three replicate readings.

From the results shown in Table 1, it was concluded that the bacterial strains isolated from pesticide contaminated soil exhibited higher emulsification activity and showed more foam stability as compared to oil contaminated soil. Strain BS-4, isolated from pesticide contaminated soil and BS-25, isolated from oil contaminated soil showed highest emulsification index in MSM as well as curd whey. Table 1 also indicated that all the 32 isolates showed better results in curd whey as compared to MSM medium. Overall BS-4 exhibited higher emulsification activity (E24%=72%) than BS-25 (E24%= 65.55) when kerosene was used as a hydrocarbons used.

In this study, among 32 bacterial cultures, 7 cultures named BS-1, BS-4, BS-5, BS-6 and BS-14 from pesticide contaminated soil and BS-21 and BS-25, from oil contaminated soil showed good zone formation indicating potent biosurfactant producing capability when curd whey was used as the growth medium. Out of 7 strains, BS-4, BS-5, from pesticide contaminated soil and BS-25, from oil contaminated soil showed greater oil displacement activity in kerosene and diesel (Table 2). Isolate 04 showed highest zone formation of 46 mm, 10 mm, 48 mm, 51 mm in kerosene, vegetable oil, petrol and diesel respectively. Isolate 25 showed zone formation of 36 mm, 8 mm, 44 mm, 48 mm in kerosene, vegetable oil, petrol and diesel respectively.

Table 2 Oil spreading assay of biosurfactant producing bacteria in MSM and curd whey.

Bacterial isolates	Zone Formation in mm (MSM/ Curd whey)			
	Kerosene	Vegetable Oil	Petrol	Diesel
Bacterial Isolates of pesticide contaminated soil				
BS-1	41/42	5/7	20/23	30/32
BS-2	30/29	7/8	36/40	28/31
BS-3	20/22	2/1	29/33	25/25
BS-4	46/46	8/10	41/48	46/51
BS-5	43/46	7/9	28/27	41/44
BS-6	30/35	4/4	26/28	39/41
BS-7	22/25	4/7	23/28	33/36
BS-8	15/20	7/9	31/34	22/20
BS-9	33/30	6/10	40/44	28/31
BS-10	31/34	2/5	29/31	40/45
BS-11	29/36	2/8	31/39	31/36
BS-12	26/28	1/4	16/21	36/42
BS-13	14/20	1/4	8/11	32/38
BS-14	38/40	6/8	37/44	42/43
BS-15	27/32	6/8	19/22	29/33
BS-16	13/20	0/1	24/28	38/38
BS-17	22/20	10/12	33/34	44/48
Bacterial isolates of oil contaminated soil				
BS-18	28/33	3/6	16/16	40/44
BS-19	30/28	1/1	15/17	31/33
BS-20	26/26	2/4	20/22	28/28
BS-21	32/35	6/9	31/36	40/44
BS-22	5/6	4/4	22/20	28/30
BS-23	17/20	6/8	16/16	33/34
BS-24	24/28	6/7	21/20	22/28
BS-25	32/36	9/8	42/44	43/48
BS-26	6/7	0/0	23/28	36/40
BS-27	18/20	½	24/24	29/31
BS- 28	22/24	8/10	19/22	19/20
BS-29	30/31	2/6	31/34	28/29
BS-30	2/4	2/2	9/8	22/23
BS-31	23/22	10/11	26/25	18/20
BS-32	22/26	7/8	29/31	31/34

Since, BS-4 and BS-25 showed highest emulsification activity and greater area for oil displacement test with positive results for drop collapse test and foam stability thus were found to be potential producer of biosurfactant and showed best results for all the above mentioned screening test. Thus BS-4 and BS-25 were selected for biosurfactant production and characterization.

The current study is focused on the isolation of biosurfactant producing bacteria from hydrocarbon polluted soil (pesticide as well as oil contaminated soil) and the potent source for highly efficient biosurfactant producers were investigated. Many published studies reported the successful isolation of biosurfactant producing organisms from soil polluted with hydrocarbons or mineral oils (Mukherjee, *et al*., 2009; Mulligan, 2005). We were successful in isolating bacteria with the ability to produce biosurfactants from the soil samples collected from garage oil contaminated soils like many other scientists have chosen the same source for soil collection, (Thavasi, *et al*., 2008 and Saravanan, *et al*., 2012).

The tests suggested by Donio, *et al*.,(2013) like drop collapse test, Oil spreading technique helped to screen lipopeptides and polymeric type of biosurfactants. Sarafin, et al., 2014, studied emulsification potential of K. marina BS-15 successfully. Selected strains also showed oil displacement activity which was confirmed by oil spreading technique (Patil *et al*., 2012). Estimation of emulsification activity is one of the principle methods for potent biosurfactant producers and emulsifying activities (E24) regulate the yield of bioemulsifier (Shoeb, *et al*., 2015). It was found that kerosene oil exhibited good response for emulsification index (Abouseoud, *et al*., 2007; Chander, *et al*., 2012). Dubey and Juwarkar *et al.*, (2001 and 2004) who used *Pseudomonas aeruginosa* as a potent strain for the production of biosurfactant when curd why was used as a growth medium. Dubey et al. (2012) also confirmed the biosurfactant production in K. turfanesis strain-J using curd whey as substrates.

E. Characterization and identification of the selected cultures

The organisms were identified by different morphological, physiological and biochemical tests. The results were tabulated (Table 3). Comparing the results with Bergey'sManual, it showed that screened and efficient biosurfactant producing isolates BS-04 and BS-25 were identified as *Kocuria rosea,* and *Micrococcus luteus/lylae,* respectively. Both strain *Kocuria rosea* (BS-4) and *Micrococcus luteus/lylae* (BS-25) found to be Gram-positive coccus and non-motile. Both the strains showed negative tests for indole production and MR. VP. *Kocuria rosea* showed negative results for VP whereas *Micrococcus luteus/lylae* showed positive results for VP. *Kocuria rosea* was negative for citrate reduction where as *Micrococcus luteus/lylae* was positive for citrate reduction. Both were urease and catalase positive. Growth of *Kocuria rosea* was observed at 4^0C, 10^0C, 20^0C, 30^0C, 37^0C and 42^0C. *Micrococcus luteus/lylae* showed growth at temperature of 20^0C, 30^0C, 37^0C and 42^0C. However *K. rosea* and *M. luteus/lylae* showed maximum growth at 37^0C and did not grow at 55^0C. The pH range of 6.0 to 11.5 supported the growth of both strains with optimum growth at pH 7.0. Both the strains tolerated 2%-10% NaCl concentrations and showed growth in all the range of 2%-10% NaCl. The two best bacterial isolates among all biosurfactant producers were selected for 16SrRNA sequencing. These strains were BS-4 and BS-25. 16S rRNA gene sequence of the strain BS-4 was most closely related to *Kocuria* sp. while BS-25 showed homology with *Micrococcus* sp. Phylogenetic analysis of the 16S rRNA sequence revealed that the isolates were *Kocuria rosea* and *Micrococcus luteus/lylae*. Phylogenetic analysis also revealed that the strains were clustered into subgroups based on their phylogenetic closeness (Figure 1 and 2).

Table 3 Morphological, physiological and biochemical characteristics of efficient biosurfactant producing isolates- BS-4 and BS-25

Test	BS-4	BS-25
Morphological tests		
Configuration	Circular	Circular
Margin	Entire	Entire
Pigment	Orange	Yellow
Shape	Cocci	Cocci
Motility	-	-
Physiological tests:		
Growth at temperatures		
4^0C	+	-
10^0C	+	-
20^0C	+	+
30^0C	+	+

37^0C	+	+
42^0C	+	+
55^0C	-	-
Growth at pH		
pH 4.0	+	+
pH 5.0	+	+
pH 6.0	+	+
pH 7.0	+	+
pH 8.0	+	+
pH 9.0	+	+
pH 10.0	+	+
Growth at NaCl (%)		
2.0	+	+
4.0	+	+
6.0	+	+
8.0	+	+
10.0	+	+
Biochemical tests		
Growth on MacConkey	-	-
Indole test	-	-
Methyl red test	-	-
Voges Proskauer test	-	+
Citrate utilization	-	+
H_2S production	-	-
Gas production	-	-
Casein hydrolysis	-	-
Gelatin hydrolysis	-	+
Starch hydrolysis	(+)	-
Urea hydrolysis	+	+
Nitrate reduction	+	-
Catalase test	+	+
Oxidase test	+	+
Acid production sugars		
Dextrose	+	-
Lactose	-	-
Sucrose	-	-

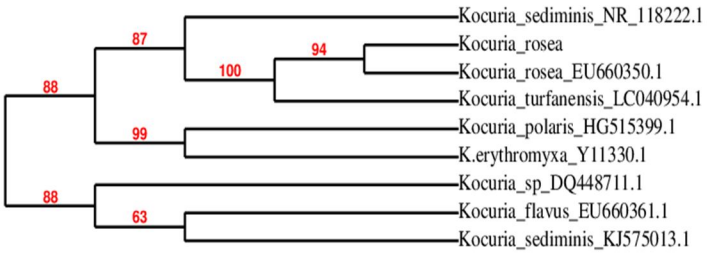


Figure 1 Phylogenetic relationships of biosurfactant producing bacterial isolate BS-04 derived from 16S rRNA gene sequence homology

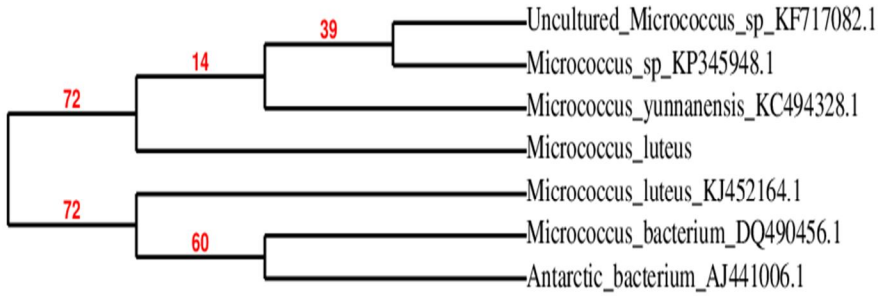


Figure 2 Phylogenetic relationships of biosurfactant producing bacterial isolate BS-25 derived from 16S rRNA gene sequence homology

F. Extraction of biosurfactant

The biosurfactant was separated without loss of its activity. The yields of biosurfactants of *Kocuria rosea* and *Micrococcus luteus/lylae* in MSM and curd whey was compared and results proved that biosurfactant yield in curd whey was more than MSM medium. The yield of *Kocuria rosea* in MSM and curd whey was 3.58 g/l and 4.28 g/l respectively, whereas *Micrococcus luteus/lylae* yielded 3.67 g/l and 4.12 g/l in MSM and curd whey respectively. This proved that curd whey produced more yield than MSM. A maximum yield of around 5.5 g/l was reported by Pruthi and Cameotra by three *Pseudomonas* species grown on n-dodecane. Rhamnolipids when grown on soy molasses yield biosurfactants in the range of 0.003- 0.213 g/l (Rashedi, *et al*., 2005). Dubey *et al*., 2014 reported that biosurfactant yield produced by isolate *Kocuria turfanesis* strain BS-J grown in curd whey was 0.98 g/l, whereas the microbial isolate belonging to the same genus *Kocuria* but different species i.e. *rosea* isolated in the present study was found to give the high yield i.e. 4.28 g/l when grown in the same medium.

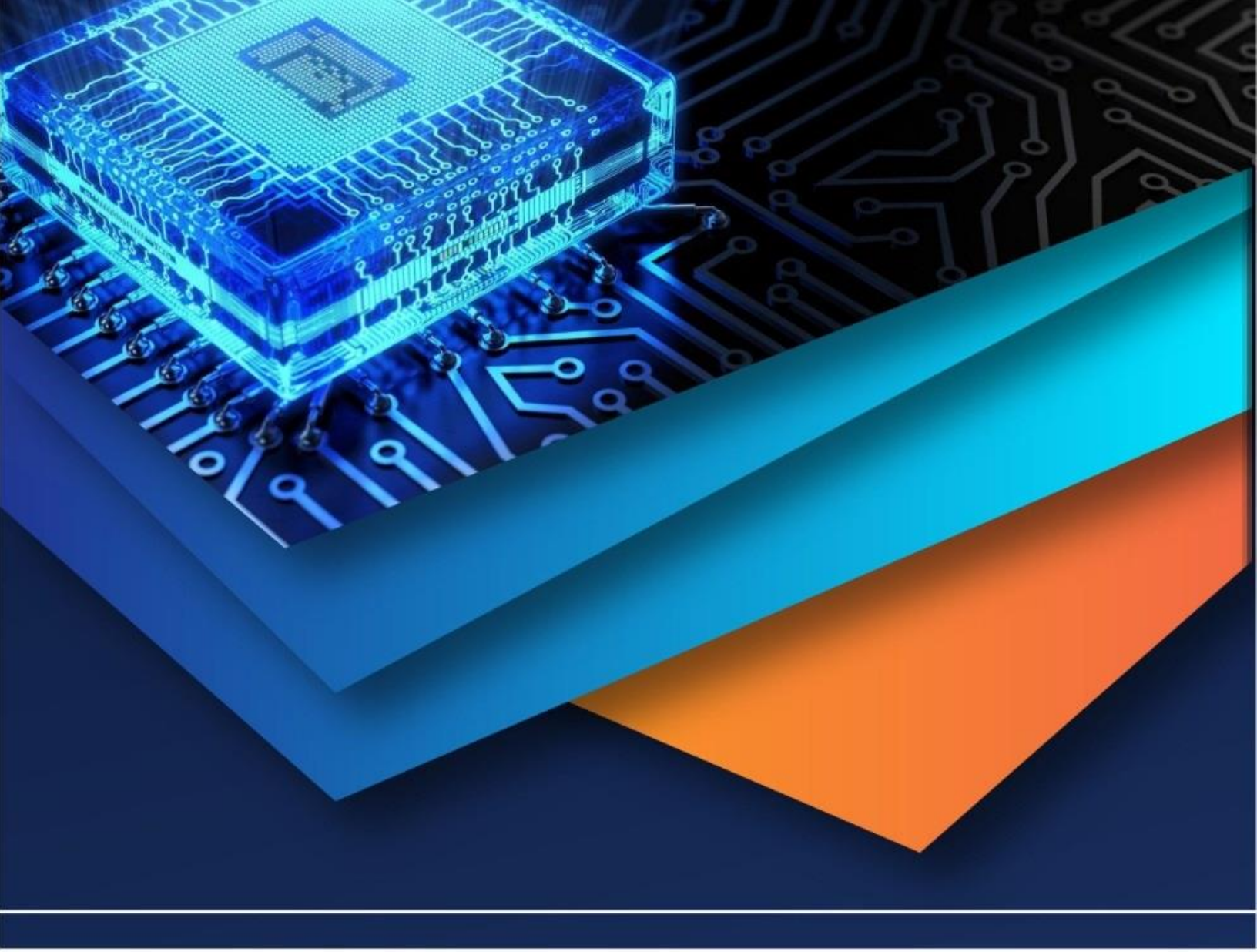
IV. CONCLUSION

This study represented comparative results of surfactant activity of the bacterial strains isolated from pesticide and oil contaminated soils. We concluded in our result that the biosurfactant produced by the isolates of from pesticide-contaminated soil gave higher potency as compared to oil-contaminated soil. The present study is an attempt to find economically cheaper sources for the large scale production of microbial biosurfactant. Results obtained in biosurfactant production with curd whey waste suggested the possibility of industrial production of biosurfactant using economically cheaper sources to reduce biosurfactant cost.

REFERENCES

[1] Abouseoud, M., Maachi, R., Amrane, A. 2007. Biosurfactant Production from olive oil by Pseudomonas fluorescens. Communicating Current Research and Educational Topics and Trends in Applied Microbiology A. Méndez-Vilas (Ed.); 340-347.
[2] Arima, K., Kakinuma, A., Tamura, G. 1968. Surfactin, a crystalline peptide surfactant produced by Bacillus subtilis: isolation, characterisation and its inhibition of fibrin clot fomation. Biochemical and biophysical research communications; 31(3): 488-494.
[3] Banat, I., Makkar, R., Cameotra. 2000. Potential applications of microbial surfactants. Applied Microbiology and Biotechnology; 53: 495-508.
[4] Batool, R., Ayub, S., Akbar, I. 2017. Isolation of biosurfactant producing bacteria from petroleum contaminated sites and their characterization. Soil and Environment; 36(1): 35-44.
[5] Bergey's Manual of Systematic Bacteriology.In Krieg NR, Holt JG, Lippincott Williams & Wilkins, Baltimore, MD, USA. 1984.
[6] Cappuccino JG and Sherman N. Microbiology-A Laboratory Manual, Addison-Wesley Longman, Inc. 1999.
[7] Chander, C.R.S., Lohitnath, T., Kumar, D.J.M., Kalaichelvan, P.K. 2012. Production and characterization of biosurfactant from Bacillus subtilis MTCC441 and its evaluation to use as bioemulsifier for food biopreservative. Advances in Applied Science Research; 3(3):1827-1831.
[8] Donio, M.B.S., Ronica, F.A., Thanga Viji, V., Velmurugan, S., Adlin Jenifer, J., Michaelbabu, M., Dhar, P., Citarasu, T. 2013. Halomonas sp. BS4, A Biosurfactant Producing Halophilic Bacterium isolated From Solar Salt Works in India and their Biomedical Importance, Springer Plus, Berlin; 2: 149.
[9] Dubey, K.V. 2014. Bioaugmentation package of egg shell based biosurfactant formulation: an innovative approach for monocrotophos degradation in soil. International journal of researches in biosciences, agriculture & technology; 2(2): 797-815.
[10] Dubey, K.V., Charde, P.N., Meshram, S.U., Yadav, S.K., Singh, S., Juwarkar, A.A., 2012. Potential of new microbial isolates for biosurfactant production using combinations of distillery waste with other industrial wastes. Journal of Petroleum and Environmental Biotechnology; S1-002.
[11] Dubey, K. V. and Juwarkar, A. A. 2001. Distillery and curd whey wastes as viable alternative sources for biosurfactant production. World Journal of Microbiology and Biotechnology; 17(1): 61-69
[12] Dubey, K. V. and Juwarkar, A. A. 2004. Determination of genetic basis for biosurfactant production in distillery and curd whey wastes utilizing Pseudomonas aeruginosa strain BS2. Indian Journal of Biotechnology; 3(1): 74-81.

[13] El-Sheshtawy, H.S., Aiad, I., Osman, M.E., AboELnasr, A.A., Kobisy, A.S. 2015. Production of biosurfactant from Bacillus licheniformis for microbial enhanced oil recovery and inhibition the growth of sulfate reducing bacteria. Egyptian Journal of Petroleum; 24(2): 155-162.
[14] Karanth, N., Deo, P., Veenanadig, N. 1999. Microbial production of biosurfactants and their importance. Current Science; 77(1):116-125.
[15] Kosaric, N. 2001. Biosurfactants for Soil Bioremediation. Food Technology and Biotechnology; 39 (4): 295–304.
[16] Macfaddin, J. F. 2000. Biochemical tests identification of medical. Bacteria. Williams and Wilkins. London.
[17] Maier, R. M. 2003. Biosurfactants: Evolution and Diversity in Bacteria. Advances in Applied Microbiology; 52: 101-121
[18] Makkar, R., Cameotra, S. 2002. An update in the use of unconventional substrates for biosurfactant production and their new applications. Applied Microbiology and Biotechnology; 58: 428-434.
[19] Meybodi, S.M., J.M. Ghayyomi, S.A. Akhavan, F. Forghani, and M.R. Kouchaki. 2013. Isolation of biosurfactant producing bacteria from petroleum contaminated soils of the Sirri Island. Petroleum Research 22(72): 110-119
[20] Mukherjee S., Das, P., Sen, R. 2009. Rapid quantification of a microbial surfactant by a simple turbidometric method. Journal of Microbial Method; 76: 38-42.
[21] Mukherjee, S., Das, P., Sen, R. 2006. Towards commercial production of microbial surfactants. Trends in Biotechnology; 24(11): 509-515.
[22] Mulligan, C.N. 2005. Environmental applications for biosurfactants. Environmental pollution; 133: 183-198.
[23] Muthusamy, K., S. Gopalakrishnan, T.K. Ravi and P. Sivachidambaram, 2008. Biosurfactants: Properties, commercial production and application, Current Science; 94: 736-747.
[24] Noordman, W.H. and Janssen, D.B. (2002) Rhamnolipid stimulates uptake of hydrophobic compounds by Pseudomonas aeruginosa. Applied and Environmental Microbiology; 68, 4502–4508.
[25] Patil, S., Aglave, B., Pethkar, A., Gaikwad, V. 2012. Stenotrophomonas koreensis a novel biosurfactant producer for abatement of heavy metals from the environment. African Journal of Microbiology Research; 6: 5173-5178.
[26] Priya, T. and Usharani, G. 2009. Comparative Study for Biosurfactant Production by Using Bacillus subtilis and Pseudomonas aeruginosa. Botany Research International; 2(4): 284-287.
[27] Pruthi, V. and Cameotra, S. S. 1995. Rapid method for monitoring maximum biosurfactant produced by acetone precipation. Biotechnology Techniques; 9: 271-276.
[28] Rodrigues, L.R., Teixeira, J.A., Mei, H.C., Oliveira, R. 2006. Physicochemical and Functional Characterization of a Biosurfactant Produced by Lactococcus lactis 53, Colloids and Surfaces B: Biointerfaces; 49: 79-86.
[29] Rosenberg, E., Ron, E. Z. 1999. High molecular-mass microbial surfactants. Applied Microbiology and Biotechnology; 52(2):154-62.
[30] Sarafin, Y., Donio, M. B. S., Velmurugan, S., Michaelbabu, M., Citarasu, T. 2014. Kocuria marina BS-15 a biosurfactant producing halophilic bacteria isolated from solar salt works in India. Saudi Journal of Biological Sciences; 21: 511–519.
[31] Sarubbo, L. A. 2006. Production and Stability Studies of the Bioemulsifier obtained from a strain of Candida glabrata UCP 1002. Journal of Biotechnology; 9: 400-406.
[32] Satpute, S. K., Bhawsar, B. D., Dhakephalkar, P. K. Chopade, B. A. 2008. Assessment of different screening methods for selecting biosurfactant producing marine bacteria. Indian Journal of Marine Sciences; 37: 243–250.
[33] Shoeb E. N., Ahmed, J., Akhter, U., Badar, K., Siddiqui, K. Ansari, F. 2015. Screening and characterization of biosurfactant-producing bacteria isolated from the Arabian Sea coast of Karachi. Turkish Journal of Biology; 39: 210-216.
[34] Thavasi, R., Jayalakshmi, S., Balasubramanian, T., Banat, I. M. 2008. Production and characterization of a glycolipid biosurfactant from Bacillus megaterium using economically cheaper sources. World Journal of Microbiology and Biotechnology; 24: 917-925.
[35] Youssef, N.H., Dunacn, K.E., Nagle, D.P., Savage, K.N., Knapp, R.M., Mclnerney, M.J. 2004. Comparison of methods to detect biosurfactant production by diverse microorganism. Journal of Microbiological Methods; 56: 339-347.



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