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RESEARCH ARTICLE

STUDY ONMICROBIAL DIVERSITY OF BIOSURFACTANT PRODUCING BACTERIA FROM CONTAMINATED ENVIROMENTAL SAMPLES.

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Abstract

Through culture enrichments we isolated nine bacterial genera: *Staphylococcus aureus* 9.1 %, *Bacillus* sp. 21.2%, *Pseudomonas aeruginosa* 27.3%, *Corynebacterium* spp., 9.1%, *Streptococcus* spp., 9.1%, *Micrococcus* spp., 12.1%, *Enterobacter* spp., 3.0%, *Proteus* spp., 6.1%, *Escherichia coli* 3.0% from numerous contaminated soils and water samples We characterized the bacterial strains that can utilize different hydrocarbons (diesel, petrol, kerosene, crude oil, vegetable oil) using βhaemolysis, methylene blue plate assay, drop collapse test, oil displacement and emulsification index; 16 isolates (66.7%) produced βhaemolysis, 6 isolates (25%) positive for methylene blue assay, 16 isolates (66.7%) collapsed in drop collapsing test. The oil spreading test on diesel, N8 (85 mm, *Bacillus* sp.) and N12 (80 mm, *Pseudomonas* sp.) displayed highest values. The emulsification index on diesel N22 (71 mm, *Proteus* sp), N7 (67 mm, *Pseudomonas* sp.) recorded the highest. All strains tested, emulsified the oil to varying degrees and their ability to produce biosurfactant ascertained.

Introduction:-

Biodiversity generally refers to the variety and variability of life on earth. It is a measure of the variety of organisms present in different ecosystems. This can refer to genetic variation, ecosystem variation, or species variation within an area or biome. Species diversity contributes to ecosystem health. Each species is like a thread holding together an ecosystem. The loss of biodiversity due to human actions has the potential to reduce multi trophic level interactions (Pinto *et al*., 2014). The variety of consortium of genes that produce biosurfactant can be studied to enhance the degradation of different components of hydrocarbons. The microbial diversity of different species of bacterial producing biosurfactant from environmental contaminated samples is a useful measure of the variety of this same gene that is responsible for biosurfactant production (Bento *et al*., 2005). Biosurfactant production is a desirable property of hydrocarbon degrading micro organism. In other to obtain efficient hydrocarbon degrading bacteria consortium and monocultures, knowledge of the diversity of the microbial community present in contaminated soil and water samples is important. The isolation of bacteria-producing biosurfactants from the environment can provide excellent materials and resources. The environmental samples are ready raw material for their isolation that is to say that biosurfactant producing bacteria are abundantly available in the environment.

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There are different screening methods for identifying biosurfactant producing bacteria. These include: β haemolysis test, methylene blue assay/Centriamide test (CTAB), drop collapsing, oil displacement test and emulsification index test. It is however, difficult to detect the type of biosurfactant produced by the microbes using a single method owing to the chemical and functional properties. In view of this, it appears that several screening methods are needed to understand the ability of a single hydrocarbonoclastic microbe in producing biosurfactant. Hence for efficient detection of potential biosurfactant producers, combinations of various screening methods are required (Satpute *et al.,* 2008). Kiran *et al.,* (2010) also suggested that the single screening method is unsuitable for identifying all types of biosurfactants, and recommended that more than one screening method should be included during primary screening to identify potential biosurfactant producers.

Emulsification of the diesel oil in water is a prerequisite that paves the way for biodegradation of environmental pollutant by many bacteria. It enhances the bioavailability of the oil and thus increases the biodegradation rate (Bredholt *et al.,* 1998; Minf *et al.,* 2011; Hassanshahian *et al.,* 2012). Very often the growth of microorganisms on hydrocarbons is accompanied by the emulsification of the hydrocarbon in the medium, and in most cases this has been attributed to the production of surface-active compounds (Desai and Banat,1997).

A wide variety of metabolic and physiological factors are required for the degradation of different hydrocarbons. All such properties are not found in one organism. Monocultures can be adversely affected by negative interactions. The best approach would be the use of a consortium of biosurfactant producing micro organisms. By selecting a consortium from various contaminated environment, the negative interactions could be minimal (Frielo *et al*., 2001). The quest for novel bacteria consortium for biosurfactant production lead to this study to identify the diversity of micro organisms from contaminated samples that produce biosurfactants.

Methodology:-

Sampling:-

The crude oil was collected from oil wells, water sample from the fish ponds in Federal Polytechnic Nekede Owerri, water samples from swimming pools, water samples from the Otamiri river, soil samples from palm oil mill, kerosene, petrol and diesel stands, soil from automobile workshop, and diary product (milk). The samples were collected in sterile vials and immediately taken to the Biology Laboratory of Federal Polytechnic Nekede Owerri for analysis.

Cultivation/Inocula Preparation:-

Samples were serially diluted, dilution of 10^{-2} to 10^{-6} plated in triplicates by spread plate method on nutrient agar medium and incubated under aerobic conditions at 37^{o}C for 24 h. Diesel/crude oil/petrol/kerosene/vegetable oil were each used as hydrocarbon source in each of the Petri-dish with the help of cotton buds and control with no hydrocarbon source was maintained. The medium was enriched with hydrocarbon source in the form of vapours. The viable cell counts of all strains were determined.

Identification Of Isolates:-

The identification of isolates were carried out using various biochemical tests to find the closest match with known bacterial genus and to assign the bacterial signature according to Bergey's manual of determinative bacteriology. The tests included gram-staining, sugar fermentation test, indole test, citrate test, spore-staining, oxidase test and catalase test.

Screening Tests:-

βHaemolysis test:-

The isolates were streaked on blood agar plates prepared with 5 ml of human blood and 4.2 g of nutrient agar powder dissolved in 150 ml of water. The blood agar plates were incubated at 30^{o}C for 24 h. The occurrence of a define clear zone around the colony is positive indication of biosurfactant production as the biosurfactant lyses the red blood cells (Morikawa, *et al.,* 2000; Youssef *et al*., 2004).

Methylene blue plate assay:-

0.1 ml of methylene blue was introduced into 150 ml of nutrient agar to make methylene blue agar. The plate were incubated at 30^{o}C for 48 h. The ability of biosurfactant producers to form clear halos in methylene blue agar plate shows the presence of biosurfactant production (Siegmund and Wagner, 1991; Lin *et al.,* 1998).

Drop collapsing test

This is a sensitive rapid method advised for screening bacterial colonies that produce biosurfactants. Drops of supernatant of biosurfactant producing colonies collapsed on oil coated surface (Lin, *et al.,* 1998; Youssef *et al.*, 2004).

Oil spreading:-

It is a method used to determine the diameter of the clear zone which occurs after adding biosurfactant containing solution on oil-water interphase. The diameter evaluation is correlated to the surface tension reduction efficiency of a given biosurfactant. The distilled water, 25 ml was taken in the large Petri dishes, 0.5 ml of oil was added onto the surface and the centre of the plates containing distilled water. Then 10 µl of the supernatant of the cultures isolated from the samples was added to the centre of the oil. The biosurfactant producing organisms displaced the oil and spread in the water. The diameter and the clear halo visualized under visible light was measured after 30 sec and results taken (Fiebigi, *et al.,* 1997; Maneerat, *et al.,* 2005).

Emulsification test:-

Emulsification capacity of the biosurfactant towards hydrocarbons (diesel, petrol, vegetable oil, kerosene, crude oil) was carried out using a mixture of 2 ml oil added to the same amount of cell free supernatant obtained after vortexing sample culture grown on nutrient broth, at high speed for 2 min and left to stand for 24 h. The emulsification index (E_{24}) was calculated as the percentage of height of the emulsified layer (mm) divided by total height of the liquid/aqueous column (mm) (Yuste *et al*.,2000*;* Schulz *et al.,* 1991).

Result:-

Table 1:- Total viable counts for all samples

S/N		Viable count	Total viable count (cfu)
1	Soil from palm oil mill	PC1 PC2 PC3 $3.9x10^5$ $2.8x10^5$ $1.2x10^4$	6.8×10^5
2	Crude oil	C1 C2 C3` 2.7×10^4 $0.7x10^4$ $1.1x10^4$	4.5×10^4
3	Water from Otmiri river	R1 R2 R3 1.0×10^4 $6.0x\ 10^4$ $4.5x\ 10^4$	11.5×10^4
4	Soil near kerosene Pump	K1 K2 K3 $1.9x\ 10^4$ $2.1x\ 10^4$ $5.0x\ 10^4$	$9.0\ X\ 10^4$
5	Soil near diesel Pump	D1 D2 K3 $1.7x10^4$ $1.8x\ 10^4$ $1.9x\ 10^4$	5.4×10^4
6	Soil near petrol pump	F1 F2 F3 $1.7x10^4$ $9.0x\ 10^4$ $1.8x\ 10^4$	12.5×10^4
7	Water from swimming pool	S1 S2 S3 2.9×10^5 $2.5x10^5$ $2.4x10^5$	7.8×10^5
8	Automobile workshop soil	AA1 AA2 AA3 $5.1x10^4$ $1.5x10^4$ $1.1x10^4$	8.7×10^4
9	Water from fish pond	FP1 FP2 FP3 $1.4x10^4$ $0.8x10^4$ $3.0x10^4$	5.2×10^4
10	Milk	M1 M2 M3 $2.0x10^4$ $1.0x10^4$ $4.7x10^4$	$7.7x10^4$
11	Abattoir	AB1 AB2 AB3 $3.0x10^4$ $2.0x10^4$ $4.7x10^4$	9.7×10^4
12	Soil from paint industry	PA1 PA2 PA3 $2.7x10^4$ $1.1x\ 10^4$ $2.8x\ 10^4$	6.6×10^4

Key: PC1, PC2, PC3 = Triplicates of soil from palm oil mill
C1, C2, C3 = Triplicates of crude oil sample
R1, R2, R3 = Triplicates of Otamiri river water sample
K1, K2, K3 = Triplicates of soil near kerosene pump
D1, D2, D3= Triplicates of soil near diesel pump
F1, F2, F3 = Triplicates of soil near petrol pump
S1, S2, S3 = Triplicates of swimming pool water sample

AA1, AA2, AA3 =Triplicates of automobile soil sample
FP1, FP2, FP3, = Triplicates of fish pond water sample
M1, M2, M3 = Triplicates of milk sample
AB1,AB2,AB3 = Triplicates of soil from abattoir
PA1, PA2, PA3 = Triplicates of soil sample from paint industry

Table 2:- Biochemical test for identification of isolates.

Sample	Isolate	Media	Morphological Characteristics	Gram stain	Spore stain	Citrate	Indole	Oxidase	Catalase	**Sugar test**				Possible Bacteria
										S	**B**	**G**	**H_2S**	
PA1, PA2, PA3	1	NA	Milkish raised non-mucoid. Circular colonies	+ve cocci	-	-	-	-	+	R	**Y**	-	-	*Staphylococcus aureus*
PA1	2	NA	Milkish flat non-mucoid colonies with undulate edges.	+ve rod	-	-	-	-	+	R	**Y**	-	-	*Bacillus* spp.
PA1, PA2	3	NA	Bluish-green pigmented non-mucoid colonies	-ve Rod	-	-	-	+	-	Y	**Y**	-	-	*Pseudomonas aeruginosa*
PA1, PA2, PC1, AC3, PB2, AC2	4	NA	Milkish raised needle pointed non-mucoid colonies	+ve cocci in chain	-	-	+	-	-	Y	**Y**	-	-	*Corynebacterium* spp.
PA2, AC2, PB3,	5	NA	Milkish flat non-mucoid colonies with rough edge	+ve cocci	-	-	-	+	-	R	**Y**	-	-	*Bacillus* spp.
PA3,PB1, PB2 PB3,PC2,PC1, AB1,AB2, AB3	6	NA	Bluish-green pigmented rhizoid-like colonies	+ve cocci	-	-	-	+	-	R	**Y**	-	-	*Pseudomonas aeruginosa*
PA3,PB1, PC3,	7	NA	Milkish flat non-mucoid colonies with undulate edges.	+ve cocci	-	-	-	+	=	R	**Y**	-	-	*Bacillus* spp.
PB2	8	NA	Milkish flat rhizoid-like colonies	+ve rod	+	-	-	-	-	R	**Y**	-	-	*Pseudomonas aeruginosa*
PB2	9	NA	Milkish flat non-mucoid colonies with zone of clearance	+ ve rod	-	-	-	-	-	R	**Y**	-	-	*Streptoccoccus spp*

Table 3:- Biochemical test for identification of isolates.

Sample	Isolate	Media	Morphological Chatacteristics	Gram stain	Spore stain	Citrate	Oxidase	Indole	Catalase	Sugar test				Possible bacteria
										S	**B**	**G**	**H_2S**	
AA3, P2	10	N.A	Milkish enlarged non mucoid colonies	+ve cocci	-	-	-	-	+	R	**Y**	-	-	*Micrococcus* spp.
AA3, PB2	11	N.A	Milkish raised non-mucoid colony	+ve cocci	-	+	-	-	-	R	**Y**	-	-	*Bacillus* spp.
AB1, AB3	12	N.A	Bluish–green pigmented colonies with zone of clearance	+ve cocci	-	+	-	+	+	Y	**Y**	+	-	*Pseudomonas* spp.
PC2	13	N.A	Golden yellow flat circular non-mucoid colonies	+ve rod	+	-	-	-	-	R	**Y**	+	-	*Streptococcus* spp.
AC3	14	N.A	Milkish enlarged flat non-mucoid colonies with irregular edges	+ve rod	+	-	-	-	+	R	**Y**	-	-	*Micrococcus* spp.
K1, K2	15	N.A	Milkish flat non-mucoid colonies with rough edges	-ve rod	-	+	-	-	+	Y	**Y**	$+^2$	-	*Enterobacteria* spp.
R1,R2,R3, M1,M3, D3, F3,C3	16	N.A	Milkish flat enlarged non-mucoid colonies with irregular edges	+ve rod	-	+	-	-	-	R	**Y**	-	-	*Micrococcus* spp.
S1,S2,S3	17	N.A	Milkish elongated non-mucoid colonies	+ve rod	-	+	+	-	+	Y	**Y**	$+^2$	-	*Proteus* spp.
D1	18	N.A	Bluish-green flat mucoid colonies with serrated edges	-ve cocci	-	+	+	-	-	Y	**Y**	+	-	*Pseudomonas* spp.
D2	19	N.A	Milkish flat enlarged non-mucoid colonies with hard-rough edges	-ve rod	-	+	-	-	-	R	**R**	+	-	*Proteus* spp.

Table 4:- Biochemical test for identification of isolates.

Sample	Isolate	Media	Morphological Characteristics	Gram stain	Spore stain	Citrate	Indole	Oxidase	Catalase	**Sugar test**				Possible Bacteria
										S	**B**	**G**	**H_2S**	
C2	20	NA	Bluish-green flat non-mucoid colonies	+ve cocci	_	_	_	_	+	Y	**Y**	+	-	*Pseudomonas* spp.
R1, R2	21	NA	Bluish-green flat non-mucoid colonies with rough edges.	+ve cocci	_	_	_	_	+	R	**Y**	-	-	*Pseudomonas* spp.
R1, FP1, M1, M3, K1, K2, F3	22	NA	Milkish flat. mucoid colonies with smooth circular edges	+ve cocci	+	_	_	_	_	Y	**Y**	-	-	*Escherichia Coli*
R3, FP3, FP1	23	NA	Milkish flat cottony non-mucoid colonies	+ve rod	+	_	_	_	+	R	**Y**	-	-	*Bacillus* spp.
R3, F3,	24	NA	Milkish flat non-mucoid colonies with serrated edges	+ve rod	+	_	_	_	_	R	**Y**	-	-	*Bacillus* spp.
R3, FP3,	25	NA	Milkish raised needle pointed non- mucoid colonies	+ve rod	_	_	_	_	+	R	**Y**	-	-	*Corynebacterium* spp.
FP2	26	NA	Milkish raised needle-like non-mucoid colonies	+ve rod	_	_	_	_	_	R	**Y**	-	-	*Corynebacterium* spp.
C1,C3,K1, F1,FP1, M3, M2.	27	NA	Golden yellow raised mucoid colonies	+ve cocci	_	+	_	_	+	Y	**Y**	+	-	*Staphylococcus aureus*
C1,C3,R1, R2,R3,M1, M2,D3,F3	28	NA	Milkish enlarged non-mucoid colonies	-ve rod	_	+	_	+	_	R	**Y**	-	-	*Micrococcus* spp.
C1, FP1, FP3	29	NA	Bluish-green flat emerged non-mucoid colonies	+ve cocci	_	+	_	+	+	Y	**Y**	-	-	*Pseudomonas aeruginosa.*
C3, R3	30	NA	Bluish-green pigmented rhizoid-like colonies.	+ve rod	_	+	+	+	+	R	**Y**	-	-	*Pseudomonas aeruginosa*
C1,R2,K2, S3,S1,FP1, M1	31	NA	Milkish-raised non-mucoid circular colonies	+ve rod	_	+	_	_	+	R	**Y**	-	-	*Staphylococcus aureus*
C2, R2, R3, M2,	32	NA	Milkish flat non-mucoid colonies with zone of clearance	+ve rod	_	_	_	_	+	R	**Y**	-	-	*Streptococcus* spp.
C3, M1, K1, K2	33	NA	Milkish flat non-mucoid colonies with rough edges.	+ve rod	+	_	_	_	+	R	**Y**	-	-	*Bacillus* spp.

Table 5:- Screening tests of bacteria isolates for biosurfactant production.

ISOLATES	HAEMOLYSIS	METHYLENE BLUE AGAR ASSAY	DROP COLLAPSING	OIL DISPLACEMENT (mm)					EMULSIFICATION INDEX E24 (%)					NAME OF BACTERIA
				DIESEL	PETROL	KEROSENE	CRUDE OIL	VEGETABLE OIL	DIESEL	PETROL	KEROSENE	CRUDE OIL	VEGRRTABLE OIL	
N1	+	-	+	20	40	45	30	5	50	50	50	50	56	*Staphylococcus aureus*
N2	+	-	+	40	30	17	40	6	50	50	50	53	45	*Bacillus* sp.
N3	-	-	+	70	36	42	54	4	50	55	53	57	50	*Pseudomonas aeurignosa*
N4	+	+	+	15	30	66	52	5	61	50	53	54	58	*Corynebacterium* sp.
N5	+	+	+	32	30	62	60	5	56	47	50	50	55	*Escherichia coli*
N6	+	-	-	16	50	10	25	5	55	53	65	52	57	*Bacillus* sp.
N7	+	-	+	15	35	30	65	5	67	50	56	50	52	*Pseudomonas aeruginosa*
N8	-	+	+	85	45	12	42	4	50	53	56	53	58	*Bacillus* sp.
N9	+	-	+	25	34	7	69	4	60	40	59	52	57	*Bacillus* sp.
N10	-	+	-	75	30	20	65	8	62	50	53	50	73	*Streptococcus* sp.
N11	+	-	+	65	40	10	50	15	63	50	44	52	46	*Bacillus* sp.
N12	-	+	+	80	42	10	52	5	50	45	54	50	50	*Pseudomonas* sp.
N13	+	-	+	15	30	35	37	5	47	44	54	72	46	*Staphylococcus* sp.
N14	-	-	-	22	42	18	30	5	59	50	65	54	48	*Micrococcus* sp.
N15	-	-	-	15	52	22	42	5	55	47	70	57	52	*Staphylococcus* sp.
N16	-	+	-	52	18	20	40	5	61	47	64	50	52	*Pseudomona aeruginosa*
N17	-	-	-	60	32	18	60	4	65	47	58	52	59	*Pseudomonas* sp.
N18	+	-	-	30	28	14	30	4	65	45	61	56	48	*Pseudomonas* sp.
N19	+	-	+	60	38	16	48	5	59	56	58	40	52	*Bacillus* sp.
N20	+	-	+	30	36	16	67	3	54	43	56	67	54	*Bacillus* sp.`
N21	+	-	+	30	35	12	57	10	61	41	61	37	45	Enterobacter sp.
N22	+	-	+	60	40	15	53	4	71	56	54	56	54	*Proteus* sp.
N23	+	-	+	13	25	10	61	17	61	53	58	72	56	*Pseudomonas* sp.
N24	+	-	-	75	50	10	62	4	55	50	54	50	52	*Proteus* sp.

Discussion:-

In the study we collected crude oil sample, water sample from Otamiri river, swimming pool, fish pond, soil samples from palm oil mill, near kerosene pump, near diesel pump, near petrol pump, automobile soil sample, paint industry and milk sample. The total viable count from Table 1 shows very high viable counts with swimming pool sample the highest (7.8×10^5 cfu) followed by palm oil mill sample (6.8×10^5 cfu). The Tables 2,3,4 are the biochemical characteristics of the isolates. We observed different genera of micro organisms which are ubiquitous as shown in Table 1 and are not restricted to a particular environmental sample. The percentage abundance of bacteria genera isolated include *Staphylococcus aureus* 9.1 %, *Bacillus* spp. 21.2%, *Pseudomonas aeruginosa* 27.3%, *Corynebacterium* spp. 9.1%, *Streptococcus* spp. 9.1%, *Micrococcus* spp. 12.1%, *Enterobacter* sp. 3.0%, *Proteus* spp. 6.1%, and *Escherichia* coli 3.0%. Several researchers (Thenmozhi and Nagasathya, 2010; El-Sheshtawy., 2013; Tambekar and Gadakh, 2013; Okore *et al.*, 2013) have isolated different *Bacillus* spp. and *Pseudomonas* spp. from crude oil and products contaminated soil and water samples. The result of this study is in agreement with the

finding. The work of (Saisa-ard *et al.,* 2013) reported the isolation of *Bacillus* spp. and *Corynebacterium* spp. from palm oil mill. This study also confirms their report. The other genera *Staphylococcus aureus*, *Proteus* spp., *Streptococcus* spp. *Escherichia coli,Micrococcus* spp. were isolated from contaminated water samples from swimming pool, fish pond, Otamiri river, abattoir and milk sample.

Based on the results on Table 5, we had the qualitative and the qualitative parts of the result. The qualitative result comprises the βhaemolysis, methylene blue agar assay and Drop collapsing method. There was a total of 16 isolates (66.7%) that produced βhaemolysis (zone of clearance around the colony); 6 isolates (25%) formed clear halos for methylene blue agar assay; 16 isolates (66.7%) collapsed on a flat surface that was coated with oil in drop collapsing test out of the 24 isolates. The isolates that had positive results on two screening methods: haemolysis and drop collapsing are N1, N2, N7, N9, N11, N13, N19, N20, N21, N22, N23. The isolates that are positive for both drop collapsing and methylene blue agar assay are N8, N12. The isolates that had a positive result for the three qualitative tests i.e. the drop collapsing, haemolysis and methylene blue agar assay are the two isolates N4-*Corynebacterium* sp. and N5- *Esherichia coli.*

Blood agar lysis has been used to quantify lipopeptide/lipoprotein (surfactin) (Lin *et al.,* 1998; Moran *et al.,* 2002; Okore *et al*., 2013) and rhamnolipids (1qbal *et al.,* 1995; Okore *et al*., 2013) and has been used to screen for biosurfactant production by new isolates. According to a research work by Youssef *et al*., (2004) β haemlysis gave a large number of false positives and negatives. That report is in contrast to the findings in this current research as 66.7% of all isolates were positive for β haemlysis test and their biosurfactant production ability was confirmed by drop collapse method that recorded also 66.7%.

The ability of biosurfactant producers to form clear halos in methylene blue agar plate assay has also been recorded by several authorities (Lin *et al.,* 1998; Govindammal and Parthasarathi, 2013; Maneerat, 2005; Siegmund and Wagner, 1991). That report is also confirmed by the finding in this report which recorded 25% positive for isolates for methylene blue agar plate assay.

Drop collapse method has been used to test for biosurfactant production by various authors (Jain *et al.,* 1991; Bodour and Miller-Maier, 1998; Youssef *et al.,* 2004; Maneerat and Phetrong, 2007; Satpute *et al.,* 2008; Karthik *et al.,* 2010; Thenmozhi and Nagasathya, 2010; Saravanan and Vijayakumar, 2012; Vanadana, 2012; Govindammal and Parthasarathi, 2013).

The quantitative part of the result comprises of the oil spreading and emulsification index method. The oil spreading test on **diesel**, the isolates that displayed highest diameter clear zone are N8 (85 mm, *Bacillus* sp.) and N12 (80 mm, *Pseudomonas* sp.); on **petrol** N15 (52 mm, *Staphylococcus* sp.), N6 (50 mm, *Bacillus* sp.) and N24 (50 mm, P*roteus* sp.); on **kerosene** N4 (66 mm, *Corynebacterium* sp.), N5 (62 mm, *Esherichia coli*); on **crude oil** N9 (69 mm, *Bacillus sp.*), N10 (65 mm, *Streptococcus sp.*); on **vegetable oil** the zones of displacement recorded for all isolates were very low but N 23 showed 17 mm (*Pseudomonas* sp.) while N 11 gave 15 mm (*Bacillus* sp.). These genera (*Proteus* spp., *Streptococcus* spp., *E.coli*, *Staphylococcus aureus*) have scarcely been reported as biosurfactant producers. In this study they equally showed positive for screening tests, exhibited various zones of displacement on hydrocarbons used and high E24. *Proteus* sp. (75 mm oil displacement test, 71% E24, +ve βhaemolysis test, +ve oil collapse test), *Streptococcus* sp.(75 mm oil displacement, +ve methylene blue test), *E.*coli (62 mm oil displacement, +ve βhaemolysis test, +ve oil collapse test, +ve drop collapse test), *Staphylococcus* sp. (72% E24, +ve βhaemolysis test, +ve drop collapse). Youssef *et al.,* (2003) in their study on comparison of methods to detect biosurfactant production by diverse microorganisms found that, the oil spreading and drop collapse methods were correlated with the ability of the cultures to reduce surface tension. The oil spreading technique measures the diameter of clear zones caused when a drop of a biosurfactant containing solution is placed on an oil water surface. Morikawa *et al.,* (2000) used this method to compare the activity of both cyclic and linear forms of surfactin and arthrofactin.

The highest emulsification index recorded by the isolates on the different hydrocarbons are: on **diesel** N22 (71 mm, *Proteus sp*), N7 (67 mm, *Pseudomonas* sp.);on**petrol,** N19 and N22 (56 mm both, *Bacillus* sp. *and Proteus* sp.), N3(55 mm, *Pseudomonas aeruginosa*); on **kerosene** N15 (70 mm, *Staphylococcus* sp.), N14 and N6 (65 mm both, *Micrococcus* sp. *and Bacillus* sp.); on **crude oil** N23 and N13 (72 mm both, *Pseudomonas* sp. and *Staphylococcus* sp.), N20 (67 mm, *Bacillus* sp.); on **vegetable oil** N10 (73 mm, *Streptococcus* sp.), N8 (58 mm, *Bacillus* sp.). *Proteus* sp., *Streptococcus* sp., *E.coli*, *Staphylococcus* sp. equally emulsified the various hydrocarbons used.

There is also a direct relationship between biosurfactant production and emulsifying activity according to Carillo *et al.,* (1996); Fiebig *et al.,* (1997); Youssef *et al.,* (2004); Tabatabaee *et al.,* (2005); Maneerat and Phetrong, (2007); Satpute *et al.,* (2008); Anandaraj and Thivakaran, (2010); Karthik *et al.,* (2010); Thenmozhi and Nagasathya, (2010); Saravanana and Vijayakumar, (2012); Vanadana, (2012); Govindammal and Parthasarathi, (2013). The result in Table 5 also confirms this report as the bacteria isolates that recorded high E24 values also showed high and positive values for the other screening tests for biosurfactant production.

Conclusion and Recommendation:-

In conclusion we isolated nine different genera of bacteria. They include *Staphylococcus aureus* 9.1 %, *Bacillus* sp. 21.2%, *Pseudomonas aeruginosa* 27.3%, *Corynebacterium* sp. 9.1%, *Streptococcus* sp. 9.1%, *Micrococcus* sp. 12.1%, *Enterobacter* sp. 3.0%, *Proteus* sp. 6.1%, and *Escherichia* coli 3.0%. The bacteria that showed high values for all screening tests were not restricted to a particular genus; virtually the genera isolated recorded high values for both E24 and oil displacement. These genera also gave positive results for βhaemolysis, methylene blue agar assay and Drop collapsing method. The diversity of these genera can be utilized in degrading different classes of hydrocarbons. Also an in-depth study on their genome and production quantities will make available such knowledge for mass production of these biosurfactant as alternative to chemical surfactants in remediation of environmental contaminants.

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