

**Table 1: Primary screening of isolates for biosurfactant production**

Hydrocarbon Contaminated (Refinery) Samples											Extreme environments									
IOCL (Mathura)		IOCL (Guwahati)		BPCL (Mumbai)		IOCL (Bongaigaon)		Reliance (Jamnagar)		Hot spring (Manikaran)		Desert soil (Pokhran)								
DCM	OSM	DCM	OSM	DCM	OSM	DCM	OSM	DCM	OSM	DCM	OSM	DCM	OSM							
TC1	++	+	GR1	++	+++	PS1	+++	++++	BRSP1	++	+++	GRTL1	-	-	HPHS1	++	+++	SB2	+++	++++
PSMR1	++	+	GR2	++	++	PSD1	-	-	BRTF1	+++	+++	GART1	-	-	HPHS2	+	++	SB1	-	-
COMR1	++	+++	GR3	-	-	MANG1	++	++	BRAT1	+	++	GMEF1	+	++						
TC2	++++	++++	GRLSC1	++	+++	IPIG1	++	+++	BRRO1	+++	++++	GCHF1	-	-						
WDM1	+	++	GRAC1	++	+	MUCK1	++	++	BRAS1	++	++	GUCK1	++	+++						
WDM2	++++	++++	GREEO	-	-	CRTB1	-	-	BRTW1	++	++									
WDM4	++	++	GREW1	++++	++++	MUCK1	++	+++	BRSOIL	+++	++									
WDM3	-	-	GRAS1	++++	++++				SLDBR	++	+++									
MRBSIT1	+++	++++	GRDS1	++++	++++															
MRBSIT2	+	++	GRSOIL1	+++	+++															
MRBRPS2	-	-																		
MRBRPS1	++	+++																		
SLDB1	+++	++++																		

**Drop collapse Method (DCM): (-) completely spherical: (+) flat: (++) moderately flat: (+++) completely flat.**

**Oil spread Method (OSM): (-) no displacement: (+) oil displacement < 1 cm in diameter: (++) 1 to 3 cm: (+++) 3-5 cm in diameter: (++++) > 5 cm in diameter.**

**Table 2: Qualitative and quantitative screening of the isolates for biosurfactant production**

S.No	Isolates	Haemolytic assay (HA)	CTAB	Surface tension (mN/m)	Emulsification index (E <sub>24</sub> ) (%)	Emulsification activity (EU/mL)
1	<i>Achromobacter</i> sp. (PS1)	+ve (β- haemolysis)	+ve	30.43 ± 0.44	69.90 ± 0.97	97.05 ± 1.62
2	<i>Ochrobactrum</i> sp. (GREW1)	+ve (α-haemolysis)	-ve	31.14 ± 0.68	59.51 ± 1.22	56.87 ± 0.89
3	<i>Bacillus</i> sp. (SLDB1)	-ve (β-haemolysis)	-ve	31.10 ± 0.71	65.23 ± 1.48	91.38 ± 1.72
4	<i>Bacillus</i> sp. (SB2)	-ve (γ-haemolysis)	-ve	28.16 ± 0.19	61.35 ± 1.15	62.98 ± 2.29
5	<i>Pseudomonas</i> sp. (MRBSIT1)	+ve (β-haemolysis)	+ve	32.24 ± 1.04	64.22 ± 1.44	70.01 ± 0.52
6	<i>Achromobacter</i> sp. (WDM1)	+ve (β-haemolysis)	-ve	34.32 ± 0.59	51.34 ± 2.26	35.91 ± 2.14
7	<i>Bacillus</i> sp. (WDM2)	+ve (β-haemolysis)	-ve	42.85 ± 1.85	22.13 ± 2.18	30.22 ± 1.10
8	<i>Bacillus</i> sp. (TC2)	+ve (α-haemolysis)	-ve	37.28 ± 1.24	21.68 ± 2.61	16.03 ± 1.08
9	<i>Bacillus</i> sp. (GR1)	-ve (γ-haemolysis)	-ve	36.65 ± 2.43	51.36 ± 1.92	13.74 ± 1.97
10	<i>Lysinibacillus</i> sp. (GRAS1)	+ve (α-haemolysis)	-ve	35.82 ± 1.62	22.15 ± 2.21	86.23 ± 0.66
11	<i>Citrobacter</i> sp. (BRRO1)	+ve (α-haemolysis)	-ve	38.40 ± 1.58	30.37 ± 1.36	39.01 ± 0.72

\*Control (Un-inoculated) (ST): 69.84 ± 2.2; Deionized water (ST): 70.31 ± 1.84  
The standard deviations presented are based on triplicate (SD, n = 3)

**Table 3: 16S-rRNA sequence results of the potent eleven bacterial isolates**

Isolates	Sample used for isolation	Bacterial isolate	Taxonomic affiliation (Class)	NCBI Accession numbers	16S-rRNA sequence comparison	
					Next relative by Genbank alignment	Similarity (%)
PS1	Oil spilled refinery soil	<i>Achromobacter sp.</i>	Betaproteobacteria	KT735240	<i>Achromobacter insolitus</i> (NR_025685)	99
GREW1	Refinery raw oil effluent	<i>Ochrobactrum sp.</i>	Alphaproteobacteria	KU321208	<i>Ochrobactrum tritici</i> (NR_114980)	100
SLDB1	Tank settled refinery sludge	<i>Bacillus sp.</i>	Firmicutes	KT735238	<i>Bacillus subtilis</i> (NR_113265)	99
SB2	Desert soil	<i>Bacillus sp.</i>	Firmicutes	KT735237	<i>Bacillus licheniformis</i> (NR_118996)	99
MRBSIT1	Bioremediation site soil	<i>Pseudomonas sp.</i>	Gammaproteobacteria	KU321209	<i>Pseudomonas aeruginosa</i> (NR_117678)	100
WDM1	Refinery waste drain	<i>Achromobacter sp.</i>	Betaproteobacteria	KT735239	<i>Achromobacter pulmonis</i> (NR_117644)	99
WDM2	Refinery waste drain	<i>Bacillus sp.</i>	Firmicutes	KT735241	<i>Bacillus subtilis</i> (NR_113265)	99
TC2	Refinery storage tank cleaning water	<i>Bacillus sp.</i>	Firmicutes	KT735242	<i>Bacillus subtilis</i> (NR_113265)	99
GR1	Refinery surge pond water	<i>Bacillus sp.</i>	Firmicutes	KT735243	<i>Bacillus aerius</i> (NR_118439)	99
GRAS1	Activated sludge	<i>Lysinibacillus sp.</i>	Firmicutes	KT735244	<i>Lysinibacillus fusiformis</i> (NR_112569)	99
BRRO1	Refinery recovered oil	<i>Citrobacter sp.</i>	Gammaproteobacteria	KT735246	<i>Citrobacter freundii</i> (NR_028894)	99

**Table 4: Evaluation of the hydrocarbon degrading potential of the biosurfactant producing isolates**

Isolate	Qualitative analysis DCPIP Method					Quantitative analysis Degradation of 2% w/v crude oil in MSM			
	Complex		Aromatic	Medium chain	Short chain	Biosurfactant production & concomitant hydrocarbon degradation		GC-FID results (% Area basis)	
	Crude	Diesel	Benzene	Decane	Hexane	Gravimetric analysis of TPH (%)	Surface tension (mN/m)	Aliphatic fraction	Aromatic fraction
<i>Achromobacter</i> sp. (PS1)	+++	++++	-	+	-	46.32 ± 4.44	32.43 ± 0.83	70.77	77.17
<i>Ochrobactrum</i> sp. (GREW1)	+++	++	-	+	-	38.93 ± 2.09	33.14 ± 0.54	79.24	72.13
<i>Bacillus</i> sp. (SLDB1)	+++	+++	-	+	-	22.58 ± 2.49	32.56 ± 0.22	87.65	67.70
<i>Bacillus</i> sp. (SB2)	++	+	-	-	+	20.91 ± 0.66	28.16 ± 1.16	82.47	73.88
<i>Pseudomonas</i> sp. (MRBSIT1)	+++	+++	+	-	+	25.19 ± 3.98	32.56 ± 0.83	93.86	41.56
<i>Achromobacter</i> sp. (WDM1)	+++	+++	-	-	-	17.50 ± 1.77	33.68 ± 1.44	50.34	49.02
<i>Bacillus</i> sp. (WDM2)	++	++	-	-	-	18.00 ± 2.22	42.85 ± 1.18	25.38	22.65
<i>Bacillus</i> sp. (TC2)	++	++	-	+	+	24.18 ± 1.10	32.37 ± 0.13	85.65	74.62
<i>Bacillus</i> sp. (GR1)	++	+++	-	-	-	23.56 ± 0.91	41.76 ± 0.52	43.90	49.25
<i>Lysinibacillus</i> sp. (GRAS1)	++	++	-	-	-	18.20 ± 2.24	58.30 ± 3.75	10.27	42.72
<i>Citrobacter</i> sp. (BRRO1)	+++	++	+	-	-	19.51 ± 2.09	45.42 ± 0.77	8.35	48.25

The symbols + and - indicate that the solution was colorless (degraded) or blue (not degraded), respectively (++++ colour change in 48h, +++ colour change in 72 h, ++ colour change in 96h, + colour change above 96 h); Control (Un-inoculated) (ST) – 59.27 ± 1.16 mN/m  
The standard deviations presented are based on triplicate (SD, n = 3)

**Table 5: Composition of fatty acids determined by GC-MS**

Fatty acid composition		Relative area percentage	
Glycolipid	Rhamnolipid (Jeneil)	<i>Achromobacter</i> sp. (PS1)	<i>Bacillus</i> sp. (SLDB1)
Decanoic acid (C <sub>10:0</sub> )	91.45 ± 0.04	88.27 ± 0.07	90.11 ± 0.07
Decenoic acid (C <sub>10:1</sub> )	0.03 ± 0.07	-	-
Dodecanoic acid (C <sub>12:0</sub> )	5.68 ± 0.03	8.25 ± 0.03	6.38 ± 0.04
Tridecanoic acid (C <sub>14:0</sub> )	0.59 ± 0.19	-	3.04 ± 0.08
Hexadecanoic acid (C <sub>16:0</sub> )	0.71 ± 0.03	3.02 ± 0.01	-
Octadecenoic acid (C <sub>18:1</sub> )	0.51 ± 0.09	-	-
Heneicosanoic acid (C <sub>21:0</sub> )	0.39 ± 0.08	1.69 ± 0.04	-
Lipopeptide	Surfactin (Sigma)	<i>Ochrobactrum</i> sp. (GREW1)	<i>Bacillus</i> sp. (SB2)
Dodecanoic acid (C <sub>12:0</sub> )	2.36 ± 0.06	-	4.93 ± 0.02
Tetradecanoic acid (C <sub>14:0</sub> )	3.21 ± 0.01	1.44 ± 0.02	-
Hexadecanoic acid (C <sub>16:0</sub> )	38.03 ± 0.06	1.26 ± 0.03	56.89 ± 0.01
Octadecanoic acid (C <sub>18:0</sub> )	14.95 ± 0.01	33.03 ± 0.09	-
Octadecenoic acid (C <sub>18:1</sub> )	-	14.84 ± 0.16	-
Nonadecanoic acid (C <sub>19:0</sub> )	39.06 ± 0.06	-	-
Docosanoic acid (C <sub>20:0</sub> )	-	17.24 ± 0.03	-
Heneicosanoic acid (C <sub>21:0</sub> )	-	32.82 ± 0.03	39.02 ± 0.03

The standard deviations presented are based on triplicate (SD, n = 3)