The potential of bacterial isolates for emulsification with a range of hydrocarbons

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Summary

A study was undertaken to investigate the distribution of biosurfactant producing, crude oil degrading bacteria in the oil contaminated environment. Our research revealed that hydrocarbon contaminated sites are the potent sources for oil degraders. Among 32 oil degrading bacteria isolated from ten different oil contaminated sites of gasoline and diesel fuel stations, 80% exhibited biosurfactant production. The quantity and emulsification activity of the biosurfactants varied. *Pseudomonas* sp. DS10-129 produced maximum of 7.5 ± 0.4 g / l of biosurfactant with corresponding reduction in surface tension from 68 mN / m to 29.4 ± 0.7 mN / m at 84 h incubation. The isolates *Micrococcus* sp. GS2-22, *Bacillus* sp. DS6-86, *Corynebacterium* sp. GS5-66, *Flavobacterium* sp. DS5-73, *Pseudomonas* sp. DS10-129, *Pseudomonas* sp. DS9-119 and *Acinetobacter* sp. DS5-74 emulsified xylene, benzene, n-hexane, Bombay High crude oil, kerosene, gasoline, diesel fuel and olive oil. The first five of the above isolates had highest emulsification activity and crude oil degradation ability and they were selected for the preparation of mixed bacterial consortium, which was also an efficient biosurfactant producing oil emulsifying and degrading culture. During this study biosurfactant production and emulsification activity were detected in *Moraxella* sp., *Flavobacterium* sp. and mixed bacterial consortium which have not been reported before.

Introduction

Biosurfactants are surface-active substances synthesised by living cells. They have the properties of reducing surface tension, stabilising emulsions, promoting foaming and are generally non-toxic and biodegradable. Interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environmentally friendly nature, possibility of large-scale production, selectivity, performance under extreme conditions and potential applications in environmental protection (1, 2). Rosenberg and Ron (3) have extensively studied the nature of microbial biosurfactants. The use of chemicals for treatment of a hydrocarbon polluted site may contaminate the environment by their by-products, whereas biological treatment may efficiently destroy pollutants while being biodegradable themselves. Biosurfactants enhance emulsification of hydrocarbons, have the potential to solubilise hydrocarbon contaminants and increase their availability for microbial degradation. Hence, biosurfactant producing microorganisms may play an important role in the accelerated bioremediation of hydrocarbon contaminated sites (3-5). These compounds can also be used in enhanced oil recovery and may be considered for other potential applications in environmental protection (5, 6). Other applications include herbicides and pesticides formulations, detergents, health care and cosmetics, pulp and paper, coal, textiles, ceramic processing and food industries, uranium ore-processing and mechanical dewatering of peat (1, 2, 7).

Several microorganisms are known to synthesize surface-active agents, most of them are bacteria and yeast (8, 9). When grown on hydrocarbon substrate as carbon source, these microorganisms synthesize a wide range of chemicals with surface activity such as glycolipid, phospholipid and others (10, 11). These chemicals are apparently synthesized to emulsify the hydrocarbon substrate and facilitate its transport into the cells.

In this paper we described the isolation and identification of several bacterial cultures from oil contaminated sites, capable of growing on hydrocarbon containing media. We also investigated the relationship between biosurfactant production and emulsification activity for various hydrocarbons.

Materials and Methods

Screening of samples

Soil samples were collected from gasoline spill (GS) and diesel fuel spill (DS) in gas station soil and wastewater (WW) samples from service stations for the isolation of oil utilizing microorganisms. Enrichment and isolation of oil degrading bacterial cultures were done using mineral salts medium (12) with Bombay High (BH) crude oil as substrate and a serial dilution agar plate technique on nutrient agar medium (Himedia, Mumbai, India).

Characterization of bacteria

The isolates were grouped to various genera as per Bergey's Manual of Determinative Bacteriology (13). These cultures were characterized depending on their morphology, gram staining, spore staining, motility, oxidase, catalase, oxidation, fermentation, gas production, ammonia formation, nitrate and nitrite reduction, indole, methyl-red, Voges-Proskauer, citrate utilization, utilization of mannitol and urea, hydrolysis of casein, gelatin, starch and lipid (14).

Growth of bacteria on BH crude oil

The bacterial cultures isolated from oil spill environment were inoculated in mineral salts medium with 1% BH crude oil as carbon source. It was kept in the shaker at 200 rpm at 30°C for a period of

seven days. The broth culture was kept undisturbed for an hour to separate the emulsion formed with crude oil at the top of the medium. The culture without oil droplets was used for bacterial growth estimation. The growth was recorded and categorized spectrophotometrically as low growth with optical density (OD) in the range 0.21-0.4, moderate growth (0.41-0.6 OD), high growth (0.61-0.8 OD) and excellent growth (0.81-1.0 OD) all measured at 620nm (15).

Selection of bacteria for surfactant production

Among oil degrading isolates 26 isolates showed biosurfactant production and therefore selected for further study (Tab. 1). They belonged to *Acinetobacter* (1), *Alcaligenes* (1), *Bacillus* (4), *Corynebacterium* (9), *Flavobacterium* (1), *Micrococcus* (1), *Moraxella* (1) and *Pseudomonas* (8). A consortium consisting of a mixture of five isolates (*Micrococcus* sp. GS2-22, *Bacillus* sp. DS6-86, *Corynebacterium* sp. GS5-66, *Flavobacterium* sp. DS5-73 and *Pseudomonas* sp. DS10-129) was also prepared and used for comparison.

Bacterial growth and biosurfactant production

A series of 500 ml flasks containing 200 ml of sterile mineral salts medium with 1% Glucose as substrate were prepared and the pH was maintained at 7.5. Each of the individual bacterial cultures and the mixed bacterial consortium were inoculated and the flasks were incubated at 30°C in a shaker at 200 rpm followed by addition of 1 % glycerol after 24 h. At every 12 h interval, biomass, biosurfactant production, surface tension and emulsification activity were measured.

Biomass estimation

The culture broth was filtered using GF/C filters, The filters were kept at 110°C for 24h. Then they were taken out and weighed. To find the net biomass, the filters were once again burnt in a furnace at 550°C and weighed. The net biomass was calculated as the difference between the two.

Biosurfactant extraction

Surface active compounds were extracted by liquid-liquid extraction (16) from 10 ml of the cell free culture broth previously acidified with 1N HCl to pH 2. Supernatant fluid was mixed with an equal volume of a chloroform: methanol (2:1) mixture. The organic extracts were concentrated by overnight drying in drying chamber at the temperature of 44 ° C and the mass of the biosurfactant was measured.

Surface tension

Surface tension was measured by drop weight method (17). A vertical fine capillary tube having round tapered nozzle was used. The liquid was drawn and passed slowly to make a fine drop, which hangs by its own weight and then falls down by gravity. The mass of a single drop from cell free culture broth was measured by the average mass of 200 drops for each sample. The following empirical formula was applied to calculate the surface tension in mN / m.

$$ST = \frac{m \times g}{3.8 \times r}$$

Where,

m = mass of single drop of liquid (mg)

r = radius of the nozzle (m)

g = gravitational force

Determination of Emulsification activity

Emulsification activity (E24) was determined by the addition of the respective hydrocarbon (xylene, benzene, n-hexane, BH crude oil, kerosene, gasoline, diesel fuel and olive oil) to the same volume of cell free culture broth, mixing with a vortex for 2 minutes and leaving to stand for 24 h. The emulsification activity was determined as the percentage of height of emulsified layer (mm) divided by total height of the liquid column (mm) (18).

Results and Discussion

The enrichment and isolation procedure resulted in 130 pure bacterial cultures able to grow in mineral salts medium (MSM) with BH crude oil as carbon source. Out of 130 isolates, 50.77%, 24.61%, 20.77% and 3.85% showed low growth (0.21-0.4 OD), moderate growth (0.41-0.6 OD), high growth (0.61-0.8 OD) and excellent growth (0.81-1.0 OD) at 620nm respectively (Tab. 1).

The isolated crude oil degraders belonged to the genera *Micrococcus*, *Corynebacterium*, *Bacillus*, *Enterobacteriaceae*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Moraxella*, *Aeromonas*, *Acinetobacter* and *Vibrio*. The biota reflects the typical heterotrophic bacteria present in soil and native genera seem to be crude oil utilizers. However, the dominant strains belonged to *Corynebacterium*, *Bacillus*, *Micrococcus* and *Pseudomonas*. The ecological studies of Marquez-Rocha et al. (19) also identified the above genera among hydrocarbon degrading microorganisms.

The addition of hydrocarbons to an ecosystem, as a result of an oil spillage, may selectively increase or decrease the size of microbial population depending upon the chemical composition of the contaminating hydrocarbons and the species of microorganisms present within the microbial community of the particular ecosystem (20). Such an event may enrich primarily for microorganisms capable of utilising the hydrocarbons and secondarily for microorganisms

capable of utilising metabolites produced by the hydrocarbon-utilising micro-organisms resulting in an increased numbers of hydrocarbon-utilising micro-organisms and associated secondary colonisers. There are numerous reports of such increases in microbial numbers following addition of hydrocarbons to a variety of microbial communities (12, 21).

Isolates *Micrococcus* sp. GS2-22, *Corynebacterium* sp. GS5-66, *Flavobacterium* sp. DS5-73, *Bacillus* sp. DS6-86 and *Pseudomonas* sp. DS10-129 had the highest growth at 30^{o}C in mineral salts medium with 1% glucose and 1% glycerol as substrates. Among these genera *Pseudomonas* sp. DS10-129 produced maximum biosurfactant of 7.5 ± 0.4 g / l at 84 h with a biomass concentration of 7.1 ± 0.6 g / l in 1% glucose + 1% glycerol as substrates and surface tension was reduced from 68 to 29.4 ± 0.7 mN / m (Tab. 2). About 0.97 - 2.7 g / l of biosurfactant production by different strains of *Pseudomonas aeruginosa* using glucose and waste fry oil as carbon source had been reported (2, 22). When compared to earlier reports *Pseudomonas* sp. DS10-129 showed higher quantity of biosurfactant production.

Among the *Corynebacterium* strains isolated, GS5-66 produced the maximum amount of biosurfactant (4.1 ± 0.6 g / l at 48 h) in glucose + glycerol and surface tension was reduced to 36.4 ± 0.2 mN / m. Similarly, Haferburg et al. (23) reported biosurfactant production by *Corynebacterium fascians* in media supplemented with yeast extract + hexadecane and kerosene, and observed a reduction in surface tension to 27.5 and 33 mN / m respectively. *Bacillus* sp. DS6-86 produced the maximum quantity of biosurfactant (2.1 ± 0.3 g / l at 48 h) with the reduction of surface tension to 31.6 ± 0.9 mN / m. Heba et al. (22) reported the production of lipopeptide biosurfactant by *Bacillus subtilis* ATCC 6633 with a reduction in surface tension of the medium to 39 mN / m. Similar reduction in surface tension was observed by Jenny et al. (24) by the lipopeptide type of biosurfactant produced by *Bacillus licheniformis*. Several authors have reported

similar activity of the biosurfactant produced by *Bacillus* sp. (25, 26). The *Acinetobacter* sp. DS5-74 produced 1.9 ± 0.2 g / l of biosurfactant in 96 h with the reduction in surface tension to 33.7 ± 0.9 mN / m. Heba et al. (22) reported lipoprotein type of biosurfactant produced by *Acinetobacter calcoaceticus* CECT 441 on olive oil and sunflower oil with reduction of surface tension to 42.5 and 38 mN / m respectively. In the earlier studies, several authors reported about the biosurfactant produced by *Acinetobacter* sp. (27).

About 2.4 ± 0.1 g / l of biosurfactant was produced by *Alcaligenes* sp. GS4-49 at 72 h with reduction in surface tension from 72 mN/m to 46.2 ± 0.7 mN / m. Dixon (28) reported that *Alcaligenes* sp. strain MM-1 produced biosurfactant similar to our findings. The production of 1.3 ± 0.2 g / l of biosurfactant by *Micrococcus* sp. GS2-22 that reduced the surface tension to 32.9 ± 0.7 mN / m was recorded at 72 h of incubation. Gutnick (29) reported the production of phospholipids and fatty acids/neutral lipid type of surfactant by *Micrococcus* sp. Other coccal forms such as *Streptococcus thermophilus* (30) produced biosurfactant, which are applied in fouling control of heat exchanger plates.

Biosurfactant production by *Moraxella* sp. DS1-13 and *Flavobacterium* sp. DS5-73 was 1.3 ± 0.1 g / l and 1.3 ± 0.7 g / l respectively. However we could not find any report on the production of biosurfactant by *Moraxella* and *Flavobacterium* in published literature. The mixed bacterial consortium produced about 4.9 ± 0.8 g / l of biosurfactant at 84 h incubation with biomass of 6.5 ± 0.4 g / l and surface tension was reduced to 34.1 ± 0.3 mN / m. When oil degraders were introduced individually, the amount of surfactant production was more when compared to the production of surfactant by mixed bacterial consortium. This may be due to the competition between the bacteria for nutrient substrate. However, biosurfactant production by mixed bacterial consortium was not reported earlier.

Petroleum hydrocarbon compounds generally bind to soil particles and are difficult to remove or degrade mainly due to limited availability to micro-organisms (31). Hence for efficient degradation, hydrocarbons should be solubilized prior to microbial degradation (32). Surfactants can emulsify hydrocarbons, thus enhancing their dispersion in water through reduction of surface tension and increased displacement of oily substances from soil particles (3, 33). Hydrocarbon contaminants may be nonavailable because of their hydrophobic nature and sorption to soil. Oberbremer et al. (34) showed that both the rate and extent of hydrocarbon degradation in soil slurry were enhanced by biosurfactants. Hence treating soil with biosurfactants will increase the availability of hydrocarbon to the degrading microorganisms, thus stimulating organic biodegradation in the soil.

The emulsification activity is an extensively used method to identify and quantify biosurfactants produced by microbial cultures. *Bacillus* sp. DS6-86 showed maximum emulsification activity on xylene (87 ± 3 %). Banat et al. (35) reported the emulsification activity on xylene during batch fermentation of pet 1006 strain in modified basal salts medium. In the earlier study *Pseudomonas* sp. MR-3 emulsified xylene to the level of 78.13% (17). *Pseudomonas* sp. DS10-129 showed 93 ± 9 % of emulsification activity on benzene and mixed bacterial consortium emulsified olive oil at the maximum of 47 ± 4 %. Heba et al. (22) reported about 61.3% emulsification activity by the glycolipid biosurfactant produced by *Pseudomonas* sp. 55T1 on olive oil.

Bacillus sp. DS2-24 showed 87 ± 6 % of emulsification activity on n-hexane. BH crude oil was emulsified to the maximum of 73 ± 6 % by *Pseudomonas* sp. DS10-129. A different strain of the same genera (*Pseudomonas* sp. MR-3) emulsified 31.70% (17). Rosenberg et al. (36) also recorded similar findings with *Arthrobacter* RAG1. Iqbal et al. (18) reported about 70% of emulsification activity on BH crude oil by *Pseudomonas aeruginosa* strain S-8. Kerosene was emulsified to 96 ± 2 % by *Pseudomonas* sp. DS4-55, while other isolates showed lesser activity, which is higher when

compared to the emulsification activity of *Arthrobacter* RAG1 (36). In our previous work, about 71.23% of kerosene was emulsified by *Pseudomonas* sp. MR-3 (17). Allen et al. (37) reported weaker emulsification activity on kerosene by some microbial isolates from subsurface soil. Johnson et al. (38) isolated *Rhodotorula glutunis* capable of producing extracellular emulsifying agent on glucose in fed batch fermentation, which emulsified n-hexadecane, xylene, kerosene and gas oil. Muriel et al. (10) observed 55% emulsification of kerosene by the cladosan biosurfactant produced by *Cladosporium resinae*. The experimental values obtained in the present investigation were higher when compared to all the earlier reports.

Microbes isolated from gasoline contaminated areas showed emulsification activity when overlaid with gasoline (37). Abu-Ruwaida et al. (39) reported the highest emulsion value (water in oil) of about 78% using Kuwait motor oil. Moreover, in the present study about 79 ± 7 % of emulsification activity on gasoline was showed by the surfactants produced by *Pseudomonas* sp. DS10-129.

Sixty two percent of emulsification activity was observed for diesel fuel by *Corynebacterium* sp. GS4-48. Willumsen and Karlson (40) found that 67% of bacterial isolates taken from polyaromatic hydrocarbon (PAH) contaminated soil were able to form detectable emulsion with diesel fuel, whereas the report of Allen et al. (37) also showed weaker emulsification activity by cultures with diesel fuel.

Members of various genera found to be capable of producing surfactants showed emulsification activity on various hydrocarbons. *Flavobacterium* sp. DS5-73 and *Micrococcus* sp. GS2-22 produced surfactants which emulsified all the hydrocarbons tested. Allen et al. (37) found that all microbial isolates from subsurface soil contaminated with unleaded gasoline showed emulsification activity when overlaid with gasoline, whereas emulsification activity by microbial cultures overlaid

with kerosene and diesel fuel were weaker. Willumsen and Karlson (40) found that 67% of their isolates were able to form detectable emulsions with diesel fuel. One might speculate that this relatively low percentage of emulsifiers among isolates from soil contaminated with PAH as opposed to soil contaminated with aliphatic hydrocarbons might indicate that growth on PAH does not require emulsification to the same extent as growth on aliphatic hydrocarbons. Alternatively, some essential growth factors for emulsification may have been lacking in their study.

Conclusion

Among the 130 bacterial isolates screened, 32 were efficient oil degraders, 80% of them were found to produce biosurfactants. Maximum of 7.5 ± 0.4 g / l of surfactant was produced by *Pseudomonas* sp. DS10-129 and minimum of 0.3 ± 0.1 g / l was produced by *Corynebacterium* sp. GS5-72. Surfactant production, biomass and emulsification activity reached the maximum at or before 96 h and was stable thereafter. No single isolate produced surfactant with maximum emulsification activity on all individual hydrocarbons tested. Biosurfactants produced by isolates such as *Micrococcus* sp. GS2-22, *Bacillus* sp. DS6-86, *Corynebacterium* sp. GS5-66, *Flavobacterium* sp. DS5-73 and *Pseudomonas* sp. DS10-129, *Acinetobacter* sp. DS5-74, *Pseudomonas* sp. GS9-119 and mixed bacterial consortium showed broad spectrum of emulsification activity with all the hydrocarbons tested. Among the biosurfactant producers, all the isolates were able to emulsify xylene and benzene. However, BH crude oil was emulsified by 88% of the isolates, n-hexane and diesel fuel by 65% and kerosene, gasoline and olive oil by 73% of them. Among the emulsifiers, more than 70% of the isolates were members of *Corynebacterium, Pseudomonas* and *Bacillus*. Our findings showed that the above oil degrading bacteria are efficient biosurfactant producers and hydrocarbon emulsifiers.

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Tab. 1 Distribution of bacteria showing different levels of growth on BH crude oil

Genera	Growth of bacteria on BH crude oil				
	Total No.	Optical Density at 620nm*			
		0.21-0.4	0.41 – 0.6	0.61 – 0.8	0.81 – 1.0
Micrococcus sp.	17	10	6	-	1
Corynebacterium sp.	45	17	17	10	1
Bacillus sp.	13	8	1	3	1
Enterobacteriaceae sp.	6	4	2	-	-
Pseudomonas sp.	16	4	2	9	1
Alcaligenes sp.	8	7	-	1	-
Flavobacterium sp.	9	7	-	1	1
Moraxella sp.	7	5	1	1	-
Aeromonas sp.	4	2	1	1	-
Acinetobacter sp.	2	1	-	1	-
Vibrio sp.	3	1	2	-	-
Total	130	66	32	27	5
Percentage	100	50.77	24.61	20.77	3.85

*

0.21 - 0.4 = Low growth 0.41 - 0.6 = Moderate growth

0.61 - 0.8 = High growth 0.81 - 1.0 = Excellent growth

Tab. 2. Maximum biosurfactant production, growth and surface tension of oil degraders

S.No.	Bacteria	Incubation (h)	Biosurfactant (g / l)	Net biomass (g/l)	Surface tension (mN / m)*	Growth on 1% BH crude oil
1	*Acinetobacter* sp.DS5-74	96	1.9[a] ± 0.2[b]	4.1 ± 0.5	33.7 ± 0.9	H
2	*Alcaligenes* sp.GS4-49	72	2.4 ± 0.1	1.8 ± 0.2	46.2 ± 0.7	H
3	*Bacillus* sp.DS1-12	48	1.8 ± 0.4	2.3 ± 0.1	33.1 ± 0.9	H
4	*Bacillus* sp.DS2-24	48	1.6 ± 0.2	2.5 ± 0.3	35.0 ± 0.2	H
5	*Bacillus* sp.DS6-86	48	2.1 ± 0.3	2.1 ± 0.2	31.6 ± 0.9	E
6	*Bacillus* sp.GS3-34	48	1.8 ± 0.1	3.7 ± 0.4	35.2 ± 0.4	H
7	*Corynebacterium* sp.DS3-37	120	2.6 ± 0.7	4.3 ± 0.6	39.7 ± 0.6	H
8	*Corynebacterium* sp.DS3-39	96	1.9 ± 0.2	3.9 ± 0.4	43.1 ± 0.3	H
9	*Corynebacterium* sp.GS5-66	48	4.1 ± 0.6	4.2 ± 0.3	36.4 ± 0.2	E
10	*Corynebacterium* sp.GS4-48	24	2.1 ± 0.3	2.5 ± 0.2	41.2 ± 0.1	H
11	*Corynebacterium* sp.GS4-52	96	2.4 ± 0.1	2.8 ± 0.3	39.3 ± 0.8	H
12	*Corynebacterium* sp.DS5-72	96	0.3 ± 0.1	2.9 ± 0.3	52.5 ± 0.1	H
13	*Corynebacterium* sp.WW1-46	24	1.4 ± 0.7	2.0 ± 0.2	45.4 ± 0.7	H
14	*Corynebacterium* sp.WW4-87	48	1.7 ± 0.2	3.5 ± 0.4	43.7 ± 0.9	H
15	*Corynebacterium* sp.WW4-92	72	1.2 ± 0.3	2.9 ± 0.2	46.8 ± 0.4	H
16	*Flavobacterium* sp.DS5-73	72	1.3 ± 0.7	3.3 ± 0.3	36.1 ± 0.2	E
17	*Micrococcus* sp.GS2-22	72	1.3 ± 0.2	1.2 ± 0.1	32.9 ± 0.7	E
18	*Moraxella* sp.DS1-13	72	1.3 ± 0.1	4.8 ± 0.5	39.5 ± 0.9	H
19	*Pseudomonas* sp.DS10-129	84	7.5 ± 0.4	7.1 ± 0.6	29.4 ± 0.7	E
20	*Pseudomonas* sp.DS1-11	48	1.7 ± 0.6	3.2 ± 0.3	38.2 ± 0.2	H
21	*Pseudomonas* sp.DS1-19	48	3.1 ± 0.8	4.5 ± 0.5	32.5 ± 0.6	H
22	*Pseudomonas* sp.DS3-38	48	2.1 ± 0.2	5.7 ± 0.6	34.2 ± 0.4	H
23	*Pseudomonas* sp.DS4-55	96	4.7 ± 0.7	4.2 ± 0.4	32.2 ± 0.2	H
24	*Pseudomonas* sp.GS4-51	72	2.6 ± 0.4	3.0 ± 0.2	32.7 ± 0.5	H
25	*Pseudomonas* sp.GS8-104	72	2.4 ± 0.1	4.4 ± 0.3	32.4 ± 0.7	H
26	*Pseudomonas* sp.GS9-119	96	4.3 ± 0.3	5.3 ± 0.5	30.6 ± 0.9	H
27	Mixed bacterial consortium	84	4.9 ± 0.8	6.5 ± 0.4	34.1 ± 0.3	E

* = Initial surface tension value 68 mN / m, [a] =average value, [b] = standard error
H = High growth (0.61 - 0.8 OD at 620 nm), E = Excellent growth (0.81 - 1.0 OD at 620 nm)

Tab. 3. Percentage of emulsification activity of the selected oil degraders on various hydrocarbons

S.No	Bacteria	Xylene	Benzene	n-Hexane	BH crude oil	Kerosene	Gasoline	Diesel fuel	Olive oil
1	*Acinetobacter* sp.DS5-74	[a]26 ± 7[b]	51± 4	48 ± 4	26 ± 2	37 ± 3	6 ± 1	9 ± 1	4 ± 1
2	*Alcaligenes* sp.GS4-49	34 ± 3	51 ± 5	27 ± 2	NE	15 ± 1	24 ± 3	9 ± 1	8 ± 1
3	*Bacillus* sp.DS1-12	4 ± 1	7 ± 1	NE	21 ± 3	NE	NE	NE	13 ± 2
4	*Bacillus* sp.DS2-24	11 ± 1	83 ± 6	87 ± 6	36 ± 2	NE	NE	NE	21 ± 1
5	*Bacillus* sp.DS6-86	87 ± 3	37 ± 2	20 ± 1	62 ± 5	11 ± 1	4 ± 2	58 ± 5	5 ± 1
6	*Bacillus* sp.GS3-34	74 ± 5	80 ± 6	NE	29 ± 4	11 ± 1	5 ± 1	4 ± 1	NE
7	*Corynebacterium* sp.DS3-37	58 ± 4	21 ± 1	NE	28 ± 2	NE	NE	NE	NE
8	*Corynebacterium* sp.DS3-39	84 ± 4	71 ± 5	NE	43 ± 3	NE	NE	NE	8 ± 1
9	*Corynebacterium* sp.GS5-66	74 ± 3	61 ± 4	70 ± 6	59 ± 4	54 ± 3	27 ± 1	39 ± 3	16 ± 3
10	*Corynebacterium* sp.GS4-48	68 ± 2	64 ± 6	73 ± 3	12 ± 1	73 ± 4	48 ± 3	62 ± 4	NE
11	*Corynebacterium* sp.GS4-52	9 ± 1	33 ± 3	NE	29 ± 3	NE	NE	NE	4 ± 1
12	*Corynebacterium* sp.DS5-72	19 ± 2	19 ± 1	37 ± 1	11 ± 1	29 ± 2	35 ± 5	NE	31 ± 3
13	*Corynebacterium* sp.WW1-46	48 ± 4	61 ± 3	7 ± 1	56 ± 4	29 ± 4	NE	6 ± 1	4 ± 1
14	*Corynebacterium* sp.WW4-87	69 ± 6	79 ± 6	52 ± 4	NE	43 ± 2	29 ± 1	33 ± 3	19 ± 1
15	*Corynebacterium* sp.WW4-92	61± 5	67 ± 7	7 ± 2	4 ± 2	14 ± 1	NE	7 ± 1	NE
16	*Flavobacterium* sp.DS5-73	3 ±1	26 ± 2	42 ± 6	68 ± 6	10 ± 1	8 ± 1	24 ± 2	21 ± 1
17	*Micrococcus* sp.GS2-22	28 ± 2	34 ± 2	19 ± 2	26 ± 2	46 ± 3	35 ± 6	27 ± 2	30 ± 3
18	*Moraxella* sp.DS1-13	14 ± 1	8 ± 1	NE	21 ± 1	19 ± 1	6 ± 1	24 ± 1	12 ± 1
19	*Pseudomonas* sp.DS10-129	74 ± 3	93 ± 9	78 ± 4	73 ± 6	89 ± 3	79 ± 7	71 ± 6	27 ± 3
20	*Pseudomonas* sp.DS1-11	29 ± 2	30 ± 2	NE	48 ± 4	4 ± 1	7 ± 1	9 ± 1	8 ± 1
21	*Pseudomonas* sp.DS1-19	71 ± 4	82 ± 6	17 ± 3	NE	31 ± 3	43 ± 4	4 ± 1	24 ± 2
22	*Pseudomonas* sp.DS3-38	78 ± 9	32 ± 1	18 ± 1	51 ± 4	NE	4 ± 1	NE	6 ± 1
23	*Pseudomonas* sp.DS4-55	75 ± 4	70 ± 6	NE	5 ± 1	96 ± 2	27 ± 3	NE	NE
24	*Pseudomonas* sp.GS4-51	9 ± 1	7 ± 1	NE	47 ± 4	NE	1 ± 0	NE	NE
25	*Pseudomonas* sp.GS8-104	37 ± 2	63 ± 5	76 ± 3	36 ± 3	49 ± 3	14 ± 1	10 ± 2	NE
26	*Pseudomonas* sp. GS9-119	52 ± 4	63 ± 4	72 ± 6	58 ± 5	84 ± 7	48 ± 3	24 ± 4	26 ± 2
27	Mixed bacterial consortium	44 ± 3	53 ± 1	66 ± 8	67 ± 6	52 ± 4	31± 2	5 ± 1	47 ± 4

[a] = Average value, [b] = Standard error NE = No emulsification detected