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Efficient breaking of water/oil emulsions by a newly isolated de-emulsifying bacterium, *Ochrobactrum anthropi* strain RIPI5-1

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ABSTRACT

Water–oil emulsions occur throughout oil production, transportation, and processing. The breaking of the water/oil emulsion improves oil quality and as a consequence chemically synthesized de-emulsifiers are commonly used in the petroleum industries. Microbial de-emulsifiers represent potential alternatives to the chemicals and may become important products for petroleum industries. The main goal of this work was isolation, identification, and characterization of an efficient de-emulsifying bacterium. Following a multi-step enrichment programme a de-emulsifying bacterium, *Ochrobactrum anthropi* strain RIPI5-1was isolated from the oil-polluted sandy bank of Siri Island, Iran. The presence of an oil phase in growth medium was found to be unnecessary for production of the de-emulsifier. The de-emulsifying activity of both the whole culture and the cells of this strain was examined using a model multiple water–crude oil (w/o/w) emulsion. This w/o/w emulsion was used for the first time in microbial de-emulsification research. Whole cells of strain RIPI5-1 exhibited high de-emulsifying activity during the late-exponential growth and stationary phases; de-emulsifying activity of the whole culture was highest during the early-exponential growth phase. The time course of de-emulsification by whole culture and whole cells of strain RIPI5-1 was investigated; the initial rate (DeI_1) of breaking of the multiple water–crude oil emulsion by whole culture and whole cells was calculated as 11% and 54%, respectively. However, overall de-emulsification ($DeI_{8.5}$) for whole culture and whole cells was calculated as 63% and 72%, respectively. A clear correlation was observed between cell surface hydrophobicity and the de-emulsifying activity of whole cells. With the water/kerosene emulsion, emulsion half-life ($t_{1/2}$) was found to be <0.5 h. The potential activity of this strain was also explained using a complex oilfield emulsion.

1. Introduction

Problems associated with the presence of water in oil include corrosion, scale formation, sludge accumulation in storage tanks, altered viscosity and flow properties, and reduced distillation efficiency [1]. The water present in oil emulsions may be from formation, through water or steam injection to improve oil recovery, or added during desalting operations. Regardless of the source of water, the emulsions have to be broken to separate out the water prior to refining.

Breaking up of the water/oil (w/o) emulsion is accomplished through a combination of physical and chemical treatment methods. The physicochemical de-emulsification processes are capital intensive; in addition a major disadvantage with the chemical de-emulsification method is disposal of the chemical de-emulsifier(s) in the aqueous phase, which have the potential to cause environmental problems. Microbial de-emulsification overcomes this disadvantage, because the biological compounds generally show both low toxicity and are readily biodegradable. Furthermore, natural products may have unique characteristics which cannot be produced by simple chemical synthesis [2]. Eighty years ago Beckman [3] issued a patent suggesting a method for treating emulsions based on the application of microorganisms. Since then several researchers have studied microbial de-emulsification [4–16] and reported some microorganisms as de-emulsifying agents. Microbial de-emulsification ability has been reported as a phenomenon associated with the whole cells, but bacterial metabolites have also been studied. The aim of this work was isolation, identification, and characterization of an efficient de-emulsifying bacterium. *Ochrobactrum anthropi* strain RIPI5-1 was isolated and identified as a new de-emulsifying strain capable of efficient breaking of (i) two experimental emulsions, a multiple water–crude oil emulsion and a water/kerosene emulsion, and (ii) a complex oilfield emulsion.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were of analytical grade. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], Tween

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Table 1
The combination of selected factors (carbon source concentration, nitrogen source, and pH) in determination of the optimal growth conditions for strain RIPI5-1.

Ethanol ($g\,l^{-1}$)			pH			Nitrogen source			Growth	
2	4	6	6.0	7.0	8.0	NH_4NO_3	NH_3^+	NO_3^{2-}	Rate[a]	Extent[b] (OD_{660})
+			+			+			0.1867	0.705
+			+				+		0.2074	0.842
+			+					+	0.1872	0.746
+				+		+			0.2345	0.98
+				+			+		0.2602	1.1
+				+				+	0.2095	0.89
+					+	+			0.2412	1.03
+					+		+		0.2416	1.00
+					+			+	0.2008	0.842
	+		+			+			0.1944	0.576
	+		+				+		0.2	0.8
	+		+					+	0.2128	0.88
	+			+		+			0.225	0.94
	+			+			+		0.2634	1.119
	+			+				+	0.2319	0.988
	+				+	+			0.2339	0.986
	+				+		+		0.2379	1.00
	+				+			+	0.2061	0.844
		+	+			+			0.1867	0.594
		+	+				+		0.195	0.785
		+	+					+	0.1723	0.675
		+		+		+			0.2185	0.905
		+		+			+		0.2334	0.98
		+		+				+	0.2115	0.89
		+			+	+			0.2232	0.95
		+			+		+		0.2201	0.915
		+			+			+	0.2005	0.86

[a] The incubation time used to calculated the growth rate was 0–41.5 h.
[b] The time used to identify the growth extent was 41.5 h of incubation.

80, and Spam 80 were obtained from Aldrich, USA. The crude oil used in this study was obtained from Siri Island, Iran. The characteristics of the crude oil are shown in Table 1.

2.2. Sampling, enrichment and isolation methods

About 140 samples including oil, oil-polluted soils, water–oil emulsions, and oily wastewater samples were collected from Iranian oilfields and food industries from which 23 mixed cultures were obtained through a multi-step enrichment programme using an enrichment medium (medium A) containing crude oil (Siri crude oil, Siri Island, Iran) as the sole carbon source; the presence of crude oil in the medium increased the potential isolation of bacteria having affinity for the w–o interface. From the mixed cultures 16 pure cultures were isolated; on the basis of their growth rate and de-emulsifying activity one strain was selected for further study. The strain was suspended in nutrient broth containing 20% (v/v) glycerol and stored at −70 °C.

2.3. Media

A medium (medium A) containing (gram per liter of distilled water) NH_4NO_3 (1.2), KH_2PO_4 (4.0), Na_2HPO_4 (6.0), $FeCl_3$ (0.001), $MnCl_2{\cdot}4H_2O$ (0.004), $MgSO_4{\cdot}7H_2O$ (0.4), $CaCl_2{\cdot}2H_2O$ (0.001), Tween 80 (1.0), and yeast extract (1.0) was used in enrichment programme. The pH was adjusted prior to autoclaving to 7.08. One ml of filter-sterilized trace element solution was added to the autoclaved medium; the trace element solution was composed of (milligram per liter of de-ionized water) $ZnSO_4{\cdot}7H_2O$ (525), $MnSO_4{\cdot}4H_2O$ (200), $CuSO_4{\cdot}5H_2O$ (705), $Na_2MoO_4{\cdot}2H_2O$ (15), $CoCl_2{\cdot}6H_2O$ (200), H_3BO_3 (15), and $NiSO_4{\cdot}6H_2O$ (27). Crude oil was added (5%, v/v) to the medium as the carbon source. Following the enrichment programme, and for the culturing of pure isolates crude oil was replaced by ethanol ($2.0\,g\,l^{-1}$) as the sole carbon source with no trace element solution (medium B). In order to optimize the growth medium several factors were changed as will be described later (medium C). Medium C without yeast extract and ethanol was used as the whole cell suspension (WCS) buffer solution.

Enriched nutrient agar (ENA) medium containing nutrient broth (13.0 g), yeast extract (2.0 g), tryptone (4.0 g), agar (15.0 g), and distilled water (966 ml) was used when necessary.

2.4. Identification of microorganism

The partial 16S rRNA gene sequences (1336 nucleotides) of the strain RIPI5-1 were determined. For completing classification of this strain a range of morphological, biochemical and physiological methods were employed.

2.5. Optimal growth conditions

To determine the optimal growth conditions a full factorial assay was carried out using three factors, carbon source concentration, nitrogen source and pH. The growth groups (27 groups; triplicate) were prepared as shown in Table 1. All inoculated growth media, together with their controls were incubated on a rotary shaker (120 rpm) at 30 °C for 48 h. During incubation at defined time intervals, growth was measured (dry cell weight, DCW) and used to calculate the growth rate. Every sample was checked for purity using ENA medium.

2.6. Preparation of whole cell suspension

Cells of the strain being studied were grown in medium C and harvested at the late exponential growth phase by centrifugation (5000 rpm, 30 min). The whole cell suspension was prepared by re-suspending the pellet in the WCS buffer solution and then adjusting the cell concentration (OD_{660}) to a suitable value.