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ORIGINAL PAPER

Chemotaxis Toward Crude Oil by an Oil-Degrading *Pseudomonas aeruginosa* 6-1B Strain

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Abstract

The chemotactic properties of an oil-degrading *Pseudomonas aeruginosa* strain 6-1B, isolated from Daqing Oilfield, China, have been investigated. The strain 6-1B could grow well in crude oil with a specific rhamnolipid biosurfactant production. Furthermore, it exhibits chemotaxis toward various substrates, including glycine, glycerol, glucose, and sucrose. Compared with another oil-degrading strain, T7-2, the strain 6-1B presented a better chemotactic response towards crude oil and its vital component, *n*-alkenes. Based on the observed distribution of the strain 6-1B cells around the oil droplet in the chemotactic assays, the potential chemotaxis process of bacteria toward crude oil could be summarized in the following steps: searching, moving and consuming.

K e y w o r d s: chemotaxis, crude oil, *Pseudomonas aeruginosa* 6-1B, swarm plate assay, modified agarose plug assay

Introduction

Microbial enhanced oil recovery (MEOR) is an environment-friendly process that may be useful to petroleum recovery. MEOR may be an efficient and inexpensive alternative method to enhance the physicochemical recovery of oil (EOR) (Kryachko 2018). Microorganisms can be used to reduce the paraffin build-up in producing wells, produce solvents or polymers above ground, and for pumping into the oil-bearing formation, as in EOR (Brown 2010). It is widely accepted that microorganisms can enhance oil recovery by their ability to produce some metabolic products, including biosurfactants. Biosurfactants are one of the most important microbial metabolic products that can reduce surface tension in oil and facilitate the emulsification of oil in with water. The latter increases the bioavailability of the residual crude oil and enhances its biodegradability (Batista et al. 2006). Furthermore, the properties of the microbes themselves, such as high cell surface hydrophobicity and motility, help them to attach to the interface and the surface of oil droplets in sandstones and carbonate strata (Rocha et al. 2020). The degradation of crude oil generally occurs at oil-water points of contact. The oil-water contact provides conditions that are the most conducive to microbial activity. The transport of hydrocarbons from the oil droplets will provide a plentiful supply of electron donors needed for metabolism, whereas inorganic nutrients required for microbial growth can be transported by water flow or diffusion to the biosphere on the oil-water contact (Head et al. 2003).

Flagellum-dependent chemotaxis is an important advantage of motile bacteria. These bacteria move through a fluid medium by rotating one or more flagella (Nakamura and Minamino 2019). Both metabolism-dependent and independent chemotaxis of *Pseudomonas* sp. toward aromatic compounds had been studied (Sampedro et al. 2015). Chemotactic bacteria, such as *Escherichia coli*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Rhodococcus erythropolis,* and others can adapt to the chemical environment by detecting changes in concentrations of certain chemicals and by changing their movement patterns essentially based on the chemical gradient present (Waite et al. 2018). The bacterial chemotaxis ability provides more opportunities for bacterial cells to move into an area with high

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concentrations of the necessary chemical attractants (Ni et al. 2020; Yang et al. 2020). Some microorganisms have evolved to use chemotaxis to resist degradation and survive conditions that lead them to utilize poisonous carbon sources, such as non-aqueous phase liquids (NAPLs), especially when there are no usable growth substrates available (Parales and Harwood 2002). Bacterial chemotaxis here is an important prelude to metabolism, as it can increase the degradation rate of NAPL-associated hydrophobic compounds (Law and Aitken 2003). Marx and Aitken (2000) have reported that chemotaxis to naphthalene by *Pseudomonas putida* G7 increased the rate of naphthalene degradation in an aqueous system in which a concentration gradient of naphthalene was imposed.

Crude oil contains NAPL-associated compounds, which are mainly composed of hydrocarbons, and aromatic compounds. It has been reported that hydrocarbon-degrading microbes migrate toward pure alkanes by chemotaxis, which may enhance the alkanes' biodegradation rate by facilitating microbial contact with the substrate (Lanfranconi et al. 2003). Favorable chemotactic properties may allow oil-degrading bacteria to efficiently detect and migrate towards oil droplets, which could be utilized as a carbon source (Meng et al. 2019). However, experimental demonstrations on chemotaxis of oil-degrading microbes towards crude oil are still limited. The potential role of chemotaxis of oil-degrading microbes in EOR has not yet received much attention. Therefore, this study investigated the chemotactic characteristics of a *Pseudomonas aeruginosa* oil-degrading strain, 6-1B, elicited by various substrates, and the chemotactic response towards crude oil via the swarm plate assay and the modified agarose plug assay. A potential chemotaxis mechanism of the 6-1B strain toward crude oil has been proposed based on the experimental results.

Experimental

Materials and Methods

Materials and media. The crude oil used in this study was light oil obtained from the Daqing Oilfield (China). It has been sterilized using high-pressure steam before use (Table S1). Tridecane and liquid paraffin were obtained from Sinopharm Chemical Reagent Co., Ltd.

Rhodococcus erythropolis T7-2 strain was cultivated in the lab (Huang et al. 2007). The bacteria were incubated in two different kinds of mineral salt media, namely medium M1 (in g/l: KH_2PO_4 0.2, Na_2HPO_4 0.6, $NaNO_3$ 2.0, $CaCl_2$ 0.01, $FeSO_4$ 0.01, $MgSO_4 \cdot 7H_2O$ 0.615, and yeast extract 0.5), and medium M2 (in g/l: Na_2HPO_4 1.5, KH_2PO_4 3.48, $(NH_4)_2SO_4$ 4.0, $MgSO_4$ 0.7, and yeast extract 0.01), respectively. The pH values of both media were adjusted to 7.2. The media were then autoclaved at 121°C for 30 min.

Screening of oil-degrading bacterial strains. The strain 6-1B, used in this study, was isolated from the oil-water of Daqing Oilfield. The strain can degrade crude oil efficiently with the production of biosurfactants. The isolation method was as follows: 10% of oil-water (w/v) was added into the mineral medium M1 supplemented with 0.2% sucrose (w/v) and 2.0% liquid paraffin (w/v) as the carbon sources and was shaken with 150 rpm at 42°C for seven days. After subculturing twice in this medium, 5% seed culture (v/v) was transferred into medium M2 supplemented with 2% liquid paraffin (w/v) as the sole carbon source. The bacteria were incubated for five days at 42°C and were subcultured for more than five times to ensure the selected strains' activity. After enriching the bacteria in the M2 medium, aliquots were diluted and smeared on Luria-Bertani (LB) agar plates to screen for single colonies. The selected colonies on the LB plate were then respectively cultured in the M2 medium at 42°C, supplemented with 2% crude oil (w/v) as the sole carbon source, and these enriched cultures were collected for subsequent testing. The degradation rate of crude oil was analyzed by a standard test method for oil and grease and petroleum hydrocarbons in water (ASTM D3921-85.1990), commonly known as the IR method. Absorbance readings were then taken by following the manufacturer's instructions, using a fixed wavelength model DM600 IR analyzer (AilunGroup, CHN). The measurement range of the IR is 0.1–10,000 ppm.

Bacterial characteristics. The biosurfactant formed by the strain 6-1B from liquid paraffin, which was the sole carbon source needed for production, was analyzed as previously reported (Patowary et al. 2017). The fermentation products of strains 6-1B and T7-2, which could emulsify diesel oil with an Emulsification Index (EI24) value of 100%, were also characterized according to respective methods well-described previously (Gandhimathi et al. 2009). In order to assess cell surface hydrophobicity (CSH), the bacterial adherence to hydrocarbon (BATH) assay was performed as described previously (Gomes et al. 2013). The taxonomy of the isolated strain 6-1B was identified according to the BLAST result of the 16S rDNA sequence with the GenBank database. The 16s rDNA was extracted from isolated colonies of the strain 6-1B and amplified using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The 16S rDNA sequence was then deposited in NCBI with an accession number of JQ012217.

Chemotaxis assays. ***Swarm plate assay.*** To characterize the chemotactic behavior of the strain 6-1B toward crude oil, the swarm plate assay was performed as previously described (Ha et al. 2014). Briefly,

cells grown on oil were collected by centrifugation at 8,000 × g for 5 min and washed twice with a chemotaxis buffer, which contained 25 mM Na_2HPO_4, 25 mM KH_2PO_4, and 0.01% yeast extract. Cells were then resuspended in the chemotaxis buffer at a final concentration of 1×10^9 CFU/ml. 0.01 ml of suspension of the strain 6-1B in the chemotaxis buffer was gently poured on the centre of the swarm medium agar plate, which contained (g/l): Na_2HPO_4 1.5, KH_2PO_4 3.48, $(NH_4)_2SO_4$ 4.0, $MgSO_4$ 0.7, 0.01% of Triton X-100, 0.01% yeast extract (v/v), and 0.01% of attractants (crude oil, liquid paraffin, and tridecane, v/v). The 0.01% of Triton X-100 (v/v) and the yeast extract were added to the swarm agar plate to improve the oil solubility and keep the mobility of cells, respectively. Overnight cultures of *P. aeruginosa* PAO-1 and *R. erythropolis* T7-2 in LB broth were used as controls. The plates were incubated at 42°C, 30°C, and 37°C, respectively, and were observed every six hours.

Modified agarose plug assay. The agarose plug assay was performed as described previously (Roggo et al. 2018), with a slight modification, by adding a coverslip on top of a concave slide to form a chamber. The cells used in these assays were harvested in the mid-logarithmic phase, washed, and resuspended with the chemotaxis buffer (with a density of about 1×10^7 CFU/ml). Plugs with crude oil sample, or melted agarose in the chemotaxis buffer (negative control), were dropped in the chamber's center. Then, 50 µl of the freshly harvested bacterial culture was then infused around the crude oil droplets. A glass coverslip was placed on top of the chamber, which was then sealed with petroleum jelly (Vaseline) to ensure that there were no air bubbles in the chamber. The movement of cells of the strain 6-1B towards the oil droplets was analyzed under the phase-contrast microscope (Olympus BH2 microscope, Japan) using the Scion Image 3b Software (Scion, Frederick, MD). The chemotaxis of the strain 6-1B was determined based on a relative velocity. A chosen 50 µm × 50 µm area was magnified 500 times to determine the relative velocity of the strain 6-1B. The chemotaxis videos were divided into frames, and an average relative velocity (derived from the average of $n = 5$ independent experiments) was determined from observed cell movements that had relatively straight trajectories. The area magnified and visualized was the interface between the oil and water; the number of cells in the field of view was counted. The changes in the pixel intensity (ranging from 0–255 PPI) reflected the bacterial density in the oil droplet's vicinity.

Morphology and image analysis. The morphology of the bacteria used in this study was observed through transmission electron microscopy (TEM, Philips EM400-ST, Japan). Chemotactic responses were observed at respective magnifications of 100×, 125×, 400×, and 500×, using a phase-contrast microscope (Olympus BH2 microscope, Japan) equipped with a CCD camera (Hitachi KP-D50 Colour Digital, Tokyo, Japan), and Axio-Vision software. The sizes of the chemotactic rings were determined using Axio-Vision software. Videos were analyzed by the method previously described (Boudko et al. 2003). The Scion Image 3b software was used to make line scan image plots.

Results and Discussion

The isolation of the strain chemotactic towards crude oil. The strain 6-1B was isolated from an oil/water sample collected in the Daqing Oilfield and can degrade crude oil upon producing a rhamnolipid biosurfactant. A 16S rRNA sequence-based phylogenetic analysis revealed that the stain 6-1B represented *Pseudomonas aeruginosa* species and was named *P. aeruginosa* 6-1B. The biosurfactant produced by the strain 6-1B was detected in the mineral salts medium M1 culture, which was supplemented with 2.0% (w/v) liquid paraffin as a carbon source. Strain 6-1B can also grow with *n*-alkanes (C_8 to C_{20}) as its sole carbon and energy source. The oil degradation rate of strain 6-1B was up to 60% (Table I).

Table I
The characteristics of the strains used in this study.

Strain characteristics	*Pseudomonas aeruginosa* 6-1B	*Rhodococcus erythropolis* T7-2	*Pseudomonas aeruginosa* PAO1
Optimum temperature (°C)	42	30	37
Fermentation product[2]	Rhamnolipid	Saccharides, protein, lipid	ND
Emulsification index (EI_{24})	100%	100%	ND
Cell surface hydrophobicity (CSH%)[1]	38%	85%	16%
Degradation range of *n*-alkenes	C_8-C_{20}	C_{12}-C_{36}	ND
Degradation rate of crude oil[3]	60.09%	75.43%	ND

[1] – bacteria were cultivated until the exponential period in LB broth
[2] – mineral salt medium M1 with 2.0% (w/v) liquid paraffin as the carbon source was used for biosurfactant fermentation
[3] – crude oil was obtained from the Daqing Oilfield, China
ND – not detected

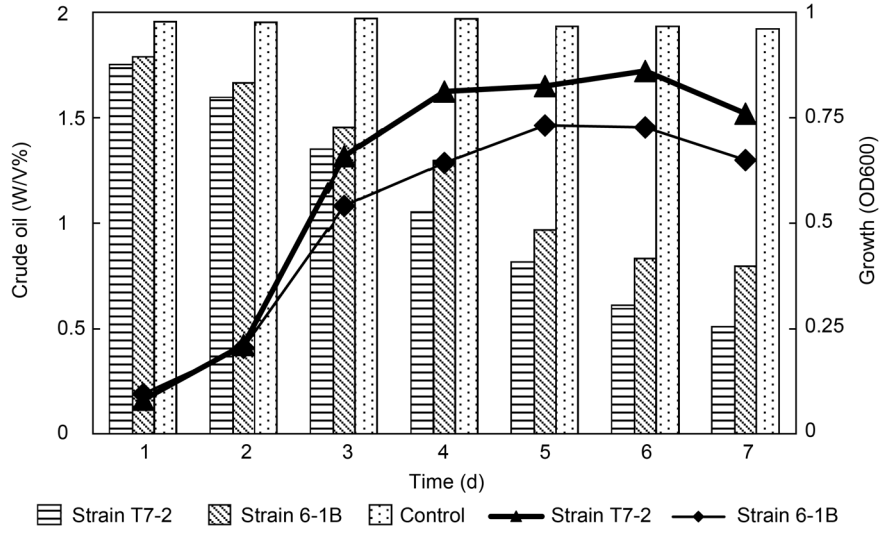

Fig. 1. Growth and degradation of crude oil by *P. aeruginosa* 6-1B and *R. erythropolis* T7-2 strains.

R. erythropolis T7-2, a non-motile strain isolated from the Daqing Oilfield, did not exhibit chemotaxis towards crude oil (Dan 2008). However, the strains T7-2 can also grow with both pure hydrocarbons as its sole carbon source (Table I). Both strains 6-1B and T7-2 were oil-degrading strains; they exhibited insignificant differences in their cell surface structures according to the results of TEM (Fig. S1), and in the cell surface hydrophobicity values (CSH%) (Table I). The TEM image showed that the strain 6-1B has a polar flagellum, while T7-2 does not. The CSH value of the strain T7-2 was 85%, which was much higher than that of the strain 6-1B (38%). Furthermore, when cultured with crude oil, the strain 6-1B showed a maximum growth at day fifth. It exhibited approximately a 15% decrease in the specific growth rate, as compared with strain T7-2.

Chemotaxis of *P. aeruginosa* 6-1B to crude oil in both the swarm plate and the modified agarose plug assays. To investigate the chemotaxis of the isolated strain towards crude oil and its components, both the swarm plate assay and modified agarose plug assay have been carried out. The results have provided several interesting insights on the chemotaxis of this oil-degrading strain towards crude oil.

First, the strain 6-1B showed chemotaxis to crude oil and its partial components, including *n*-alkanes. In the swarm plate assay, the enriched culture of the strain 6-1B was gently poured on the centre of swarm plates containing attractants, which were also the carbon source. In these plates, actively metabolizing the strain 6-1B generated a gradient of the carbon sources present. Chemotactic bacteria and their growth correspondingly following such a gradient resulted in the so-called swarm rings' formation. Therefore, the swarm rings' presence indicated the strain 6-1B's chemotactic behavior towards all the three attractants, including crude oil, liquid paraffin, and tridecane (Fig. 2). Bacterial growth, typical of a chemotactic response to a self-generated gradient, was observed in tridecane-

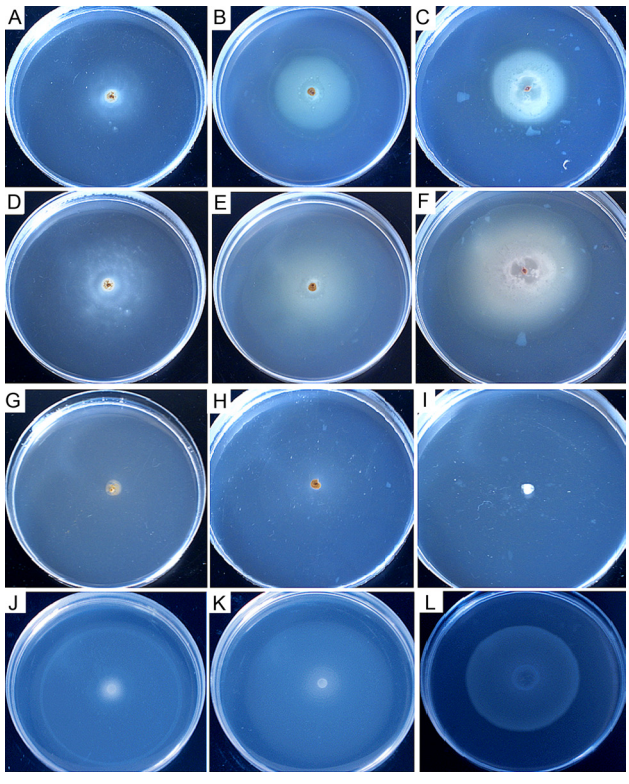

Fig. 2. Chemotaxis of the strain 6-1B towards tridecane, liquid paraffin, and crude oil in the swarm plate assay.

The chemotaxis of the strain 6-1B towards 0.01% of tridecane (A) and (D), liquid paraffin (B) and (E), crude oil (C) and (F), and the chemotaxis buffer without attractant (G) were determined, respectively. The chemotactic responses of the control strain *R. erythropolis* T7-2, and *P. aeruginosa* PAO-1 to crude oil are shown in (H) and (I). Among them, Fig. 2A, 2B, and 2C were photographed after 24 hours of cultivation; Fig. 2D, 2E, 2F, 2G, 2H, and 2I were taken after 48 hours of incubation. The chemotactic responses of the strain 6-1B towards sucrose, glycine, glycerol after 24 hours of cultivation are also shown in Fig. 2J, 2K, and 2L separately.

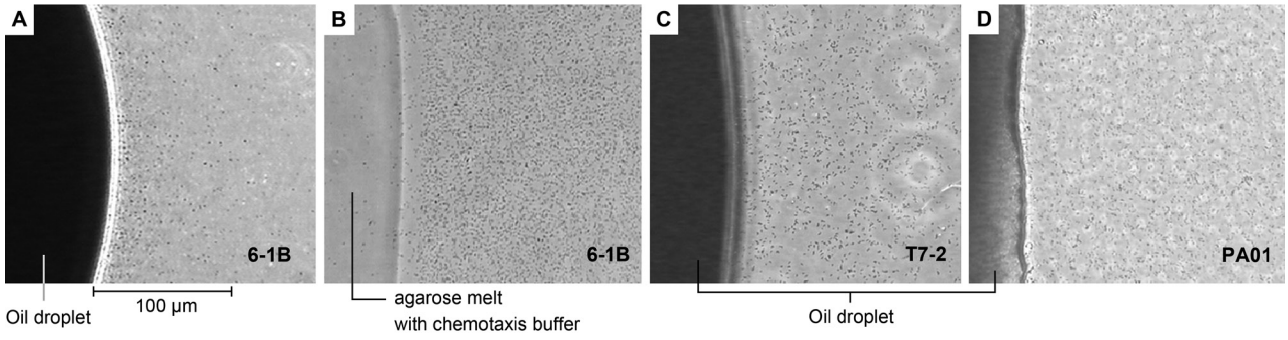

Fig. 3. The chemotactic responses of the strain 6-1B (A), T7-2 (C), and PAO1 (D) towards crude oil via modified agarose plug assay. The chemotaxis of the strain 6-1B towards the chemotaxis buffer served as the negative control (B).

(Fig. 2A and 2D), liquid paraffin- (Fig. 2B and 2E), and crude oil- (Fig. 2C and 2F) containing plates after 24 or 48 hours of incubation.

Similarly, the results of the modified agarose plug assay demonstrated that the strain 6-1B, which was in the exponential phase of growth, retained a strong ability to move and adapt its chemotactic ability towards crude oil, as shown by the chemotactic ring formed around the oil attractant (Fig. 3A). Several studies have examined the chemotactic responses of bacteria toward their carbon source and demonstrated that chemotaxis enables bacteria to position themselves in environments favorable for survival (Pandey and Jain 2002; Pedit et al. 2002; Zheng et al. 2020). Zheng et al. (2020) determined that chemotactic motility plays a crucial role in microbial oil degradation. Microbes' chemotactic motility could cause a continuous bump onto the oil drop, resulting in mechanical disruption of the oil-water interface and dispersion of the oil components into the surrounding motility buffer. This phenomenon likely created a concentration gradient, which chemoattracted more bacteria toward their carbon source. Furthermore, chemotactic responses to the Daqing Crude oil components indicated that the strain 6-1B is chemotactic to *n*-alkenes, such as dodecane, tridecane, tetradecane, pentadecane, hexadecane. Simultaneously, no chemotactic response towards aromatic hydrocarbons naphthalene, diphenyl, and sulfur was observed (Table II). However, the chemotaxis ring formed around some *n*-alkanes, such as tridecane, was not as apparent as those formed around the liquid paraffin and crude oil (Fig. 2). This difference may be due to many hydrocarbons in the liquid paraffin and crude oil plates. Another potential explanation could be related to the relatively low extent of tridecane's degradability (56%). The chemotaxis ring observed around the liquid

Table II
Chemotaxis responses of the different strains to various components of the Daqing crude oil and their respective relative degradation rates.

Attractants[1]	*Pseudomonas aeruginosa* 6-1B		*Rhodococcus erythropolis* T7-2		*Pseudomonas aeruginosa* PAO-1	
	Chemotaxis response[2]	Oil degrading rate (%)[3]	Chemotaxis response[2]	Oil degrading rate (%)[3]	Chemotaxis response[2]	Oil degrading rate (%)[3]
Dodecane	+	63.22	–	78.17	–	ND
Tridecane	+	56.18	–	75.62	–	ND
Tetradecane	+	54.28	–	67.57	–	ND
Pentadecane	+	57.97	–	62.84	–	ND
Hexadecane	+	55.54	–	59.73	–	ND
Liquid paraffin	+	58.13	–	65.11	–	ND
Crude oil	+	60.09	–	75.43	–	ND
Naphthalene	–	ND	–	ND	–	ND
Diphenyl	–	ND	–	ND	–	ND
Sulfur	–	ND	–	ND	–	ND

[1] – all the attractants was tested at a concentration of 0.01% (w/v)
[2] – chemotactic response was tested by the swarm plate assay
[3] – the degradation rate of crude oil was tested by an IR analyzer
\+ – cells are chemotactic towards the substance
– – there is no chemotactic response
ND – not detected

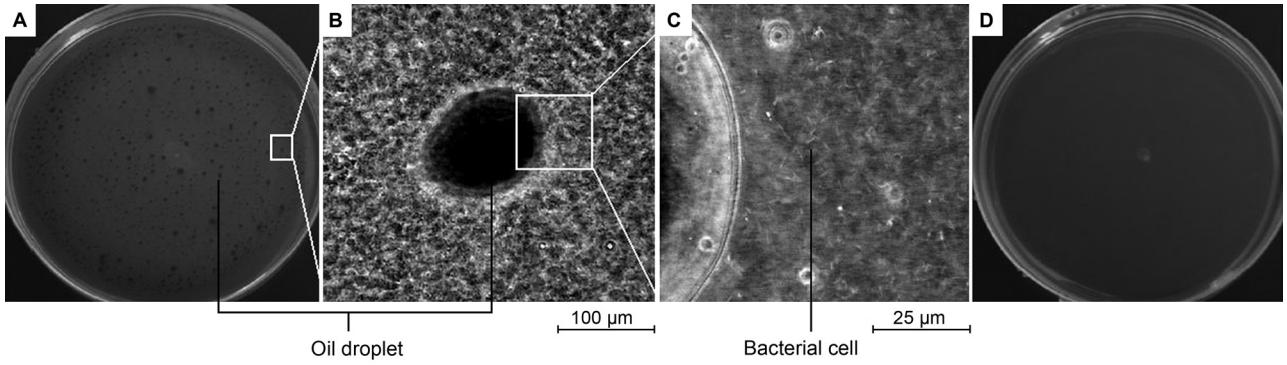

Fig. 4. The visualization of chemotaxis rings in the swarm plate using microscopy.
The chemotaxis of the strain 6-1B toward crude oil (0.1%) visualized using a phase-contrast microscope (Olympus BH2 microscope, Japan) with magnifications of 0× (A), 125× (B) and 500× (C), respectively; the control swarm plate which contained the same composition except for crude oil, visualized at a magnification of 0× (D).

paraffin was evident and similar to that of the crude oil, probably because liquid paraffin is mainly composed of linear *n*-alkanes ranging from C_{12} to C_{18}. The strain 6-1B did not migrate toward other Daqing Crude oil compositions, such as aromatic hydrocarbons, naphthalene, diphenyl, and sulfur within the same timeframe. We proposed that this oil-degrading strain showed chemotaxis to crude oil mainly because it has chemotactic responses to linear hydrocarbons, which bacteria can use as growth substrates. We, therefore, hypothesize that oil-degrading strains show chemotaxis towards *n*-alkanes, which is a component of the crude oil, and that the phenomenon of chemotaxis towards crude oil is, in fact, the chemotactic response towards the hydrocarbons present in the crude oil.

Another notable insight into the degrading strain's displayed chemotaxis is that not all oil-degrading strains exhibit the chemotactic response towards crude oil. Our study also evaluated the chemotactic process of another oil-degrading strain *R. erythropolis* T7-2 and found that T7-2 showed chemotaxis towards crude oil is neither the swarm plate assay (Fig. 2H) nor the modified agarose plug assay (Fig. 3C). A standard laboratory strain *P. aeruginosa* PAO-1, which could neither degrade nor chemotaxis toward crude oil, was used as a control (Fig. 2I and 3D). These results suggest that not all oil-degrading bacterial strains are chemotactic towards the crude oil. Some strains were not even mobile and failed to form chemotactic rings, although they could efficiently degrade crude oil. These microorganisms usually have unique properties, such as a high cell surface hydrophobicity observed for Gram-positive bacteria and unique cell surface structures in other bacteria (Zita and Hermansson 1997; Liu et al. 2004). As previously reported, bacterial adhesion is also a strategy for oil-degrading bacteria to attach to the surface of crude oil, which mainly depends on the bacterial hydrophobic property (Bruinsma et al. 2001). The *R. erythropolis* T7-2 strain, for example, known for its high cell surface hydrophobicity, displays mycolic acids with carbon chain lengths ranging from 27 to 54 on its cell surface. These observations provide a foundation for biological applications in demulsifying crude oil emulsions in water obtained from oil fields. The T7-2 strain cells' surface is strongly hydrophobic (Table I), which allowed this strain to come into contact with organic compounds, such as crude oil, more efficiently. The T7-2 strain was shown to be able to use *n*-alkanes from C12 to C36, with about a 60% extent of hexadecane degradation (Huang et al. 2007). Shaking facilitated fermentation of the oil degradation medium of T7-2 improved the adherence of bacterial cells to the crude oil, as well as the rate of degradation. This type of microorganism, which is used in EOR might depend mainly on their biosurfactant productivity and hydrophobic properties *in situ*.

Furthermore, our results support the existence of chemotactic migration of the strain 6-1B towards crude oil. To analyze the chemotactic process of *P. aeruginosa* 6-1B further, we modified the swarm plate to an oil plate by maintaining the same composition while increasing the concentration of crude oil to 0.1% (Fig. 4). The higher crude oil concentration caused many small oil droplets on the swarm plate. A faint ring was formed on the oil plate (Fig. 4A). The ring's edge was excised and observed under the microscope, which revealed that many bacterial cells surrounded the oil droplet (Fig. 4B and 4C). Based on the 16S rRNA sequencing analysis, cells surrounding the oil droplet were *P. aeruginosa* 6-1B (100% identity), which demonstrated the existence of chemotactic migration by the 6-1B strain towards crude oil. Similar results have been obtained in the modified agarose plug assay (Fig. 3A and 5A). The maximum cell density was found at the water and oil interface (Fig. 5C); there was a rising trend in cell density as we observed nearer to the interface, which we attributed mainly to a large number of highly motile strain 6-1B that accumulated in the interface around

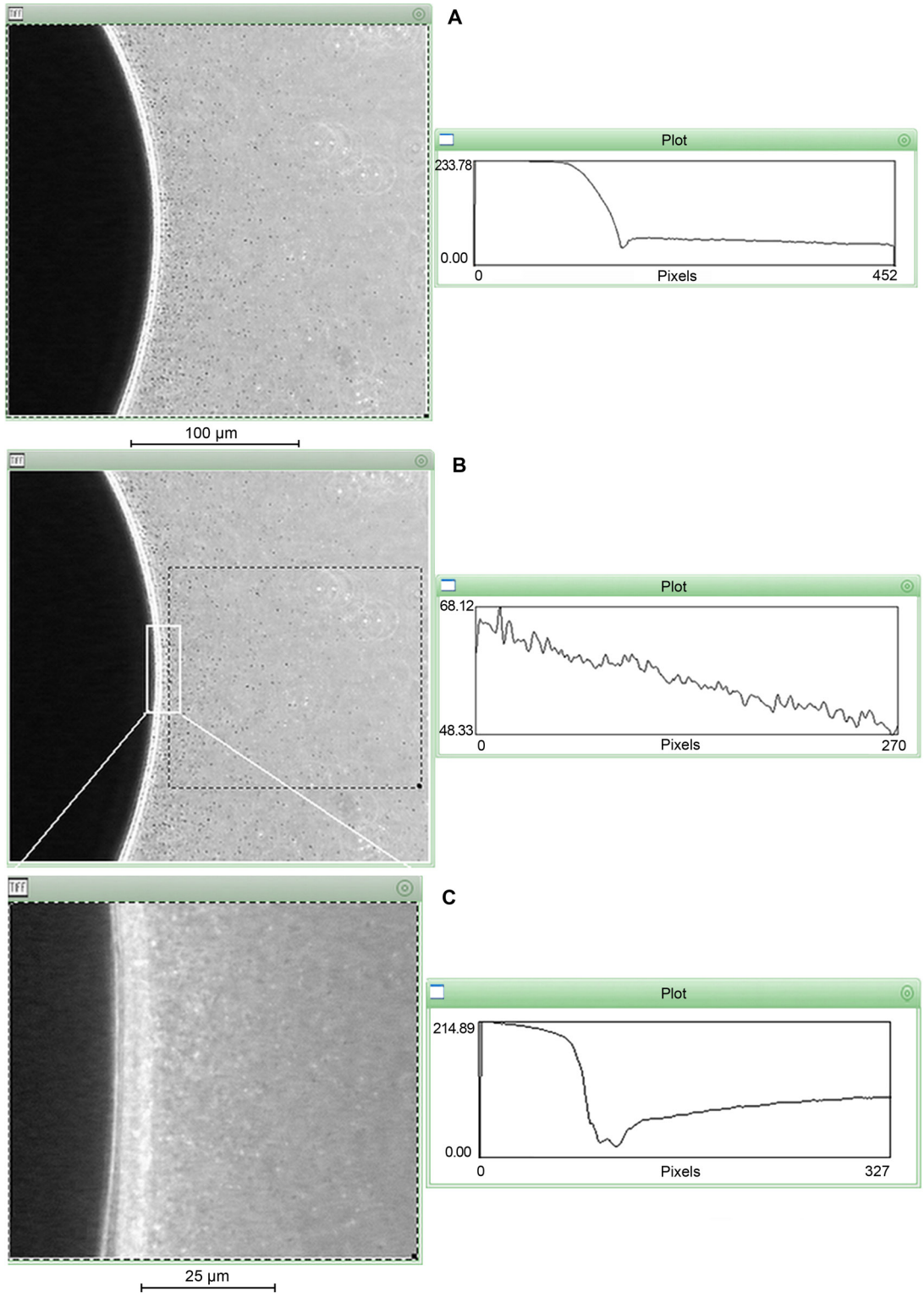

Fig. 5. *P. aeruginosa* 6-1B cells' distribution around the crude oil droplet.

The strain 6-1B movement towards the oil droplets was analyzed under the phase-contrast microscope (Olympus BH2 microscope, Japan) using the Scion Image 3b Software (Scion, Frederick, MD). A) The chemotactic trend of the strain 6-1B toward crude oil (125× magnification); B) the chemotactic trend of the dashed area; C) the chemotactic trend of the strain 6-1B toward crude oil of the area of the white line in Fig. 5B (400× magnification).

the oil droplet when they were observed using negative phase-contrast microscopy.

In contrast, cells of the strain T7-2 were uniformly distributed around the oil droplet and tumbled *in situ*, with no cells moving forward (Fig. 3C). The non-degrading PAO1 cells also moved randomly, showing no directionality in their movements (Fig. 5C). Based on the evidence listed above, we can conclude that the 6-1B strains present chemotactic migration towards the crude oil.

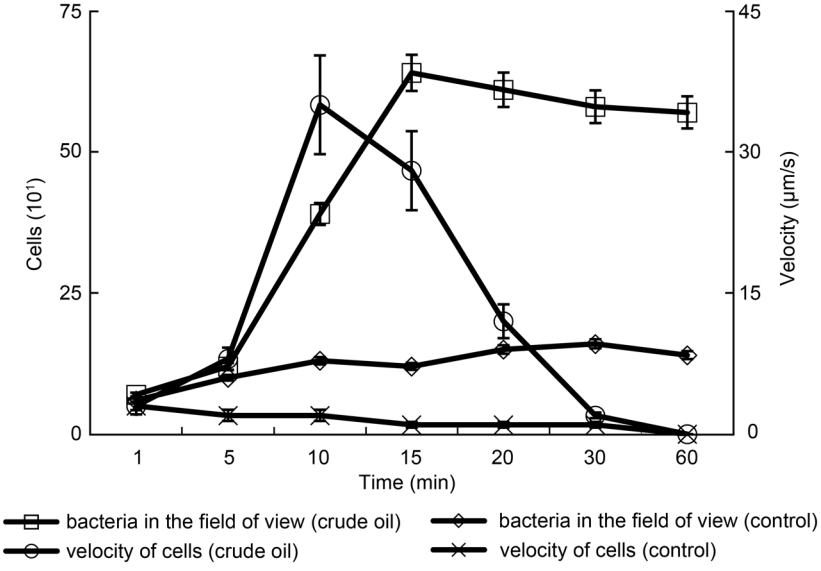

Fig. 6. The number of chemotactic cells (left y-axis) and chemotactic velocity curves (right y-axis) of *P. aeruginosa* 6-1B toward the crude oil.

The control chamber was treated without crude oil, which served as the chemoattractant in the other setups. A chosen 50 μm × 50 μm area was magnified 500 times for the determination of cell velocity. The chemotaxis videos were divided into images, and an average velocity was determined from observed cell movements that had relatively straight trajectories. Data represent the averages and the standard deviations of five independent experiments.

Potential chemotactic process of *P. aeruginosa* 6-1B toward the crude oil. Although chemotaxis rings around NAPL-related compounds observed by microscopy had been reported (Vardar et al. 2005), the detailed description of that process was still limited. Here, we characterized the chemotaxis process of the strain 6-1B toward the crude oil (Fig. 6). In the first 5 minutes, most of the cells tumbled *in situ* and did not move at all, while only a few cells moved towards the oil droplet at a low velocity (about 3–5 μm/s). During the following 10 minutes, the cells rapidly swarm toward the crude oil droplet interface, with a velocity reaching up to 35 μm/s. The speed changed as time passed, as the number of cells around the oil and near the interface increased. The velocity of the cells declined quickly after 30 minutes. The cells then stopped swimming toward the oil chemoattractant and tumbled *in situ* for several days. According to the above results, the potential chemotaxis process of the strain 6-1B toward crude oil could be summarized in the following steps: searching for the attractant crude oil, moving toward the attractant crude oil, and consuming (degrading) the crude oil. In general, the strain 6-1B can successfully reach the oil-water interface and degrade them by achieving these steps. This chemotactic mechanism may provide the strain 6-1B a greater chance of contacting and utilizing the crude oil as a carbon and energy source.

The chemotactic response to some attractant chemicals is of great advantage for bacterial survival. Bacterial chemotaxis can usually form a dense distribution of cells around the oil quickly, and it is an effective way to maintain hydrocarbon bioavailability (Pandey and Jain 2002). Chemotaxis provides a more active process to gain proximity to a liquid hydrocarbon source to enhance cell growth and increase the apparent dissolution rate of the hydrocarbon. Contact between a bacterial cell and a target hydrocarbon can significantly increase hydrocarbon diffusion rate into the cell (Meng et al. 2019). To efficiently metabolize the crude oil as a carbon source, the strain 6-1B should migrate closer to the oil and water interface via chemotaxis towards the increased concentrations of crude oil compounds dissolved in the aqueous phase. In this manner, the strain 6-1B can efficiently utilize the hydrocarbons at the oil-water interface, improving their growth. Their ability to produce the rhamnolipid biosurfactant will also increase, improving the crude oil's solubility, decreasing the surface tension and the interfacial tension between oil and water. The biosurfactant was shown to play an essential role in microbial degradation of the crude oil by facilitating the utilization of crude oil dissolved in an aqueous phase (Lee et al. 2018). The rhamnolipids improve the miscibility of hydrocarbons and crude oil in water, contributing to the degradation of crude oil significantly.

Conclusion

Although chemotaxis towards pure hydrocarbons has been demonstrated, the research on bacterial chemotaxis towards crude oil is still limited. In this study,

an oil-degrading strain of *P. aeruginosa,* named 6-1B, has been found to have chemotactic activity towards crude oil and its gradient *n*-alkenes such as dodecane, tridecane, tetradecane, pentadecane, and hexadecane, as demonstrated by the use of two different chemotactic assays. Moreover, according to the different distributions of cells around the crude oil, the potential chemotaxis process of the strain 6-1B toward crude oil is proposed as the following steps: searching, moving and consuming. Thus, it may be assumed that the chemotaxis-dependent movement of the strain 6-1B enhances the chance of bacteria to reach contact with the crude oil, resulting in the improvement of the degradation of crude oil. Subsequently, the produced biosurfactant during degradation could then be used to enhance oil recovery when this process is applied in underground oil wells.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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