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Degradation of petroleum hydrocarbons (C6–C40) and crude oil by a novel *Dietzia* strain

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ABSTRACT

A novel bacterial strain, DQ12-45-1b, was isolated from the production water of a deep subterranean oil-reservoir. Morphological, physiological and phylogenetic analyses indicated that the strain belonged to the genus *Dietzia* with both *alkB* (coding for alkane monooxygenase) and CYP153 (coding for P450 alkane hydroxylase of the cytochrome CYP153 family) genes and their induction detected. It was capable of utilizing a wide range of *n*-alkanes (C6–C40), aromatic compounds and crude oil as the sole carbon sources for growth. In addition, it preferentially degraded short-chain hydrocarbons (⩽C25) in the early cultivation phase and accumulated hydrocarbons with chain-lengths from C23 to C27 during later cultivation stage with crude oil as the sole carbon source. This is the first study to report the different behaviors of a bacterial species toward crude oil degradation as well as a species of *Dietzia* degrading a wide range of hydrocarbons.

1. Introduction

Microorganisms able to utilize saturated hydrocarbons (*n*-alkanes) are widely distributed in nature (Atlas and Atlas, 1991; Zhang et al., 2011), comprising members of at least 60 genera of aerobic bacteria and five genera of anaerobic bacteria (Prince, 2005), including *Acinetobacter* (Sakai et al., 1994), *Rhodococcus* (van Hamme and Ward, 2001), *Alcanivorax* (Liu et al., 2010) and *Pseudomonas* (Zhang et al., 2011). Among these bacteria, *Dietzia* species are considered to be ubiquitous, having been isolated from diverse environments worldwide, such as soda lakes (Duckworth et al., 1998), oil fields (Borzenkov et al., 2006), soil, deep-sea sediments (Colquhoun et al., 1998), decomposing reed rhizomes (Borsodi et al., 2005), skin and the intestinal tracts of marine fish (Yumoto et al., 2002). Several of the *Dietzia* species described to date have been shown to degrade aliphatic hydrocarbons (Yumoto et al., 2002; Nesterenko et al., 1982; Rainey et al., 1995; Kumar et al., 2011). For example, *Dietzia maris* DSM 43672^T was found to grow with C6 to C17, C19 and C23 *n*-alkanes (Rainey et al., 1995), while *Dietzia psychralcaliphila* utilized the *n*-alkanes C13, C15, C16, C20, C24 and pristane, but not C10 or C32 (Yumoto et al., 2002). A recent publication showed that *Dietzia* sp. E1 could degrade *n*-alkanes with the chain length ranging from C6 to C30 (Bihari et al., 2010). In addition, *Dietzia* strains have been reported to degrade aromatic compounds, including naphthalene (von der Weid et al., 2007), phenanthrene (Al-Awadhi et al., 2007), benzoate (Maeda et al., 1998), carbazole, quinoline, fluoranthene (Kumar et al., 2011) and toluene (von der Weid et al., 2007).

Although many studies have reported the abilities of bacteria to degrade hydrocarbons with different carbon numbers, most bacteria can use only a narrow range of hydrocarbons. For example, *Bacillus stearothermophilus* is only capable of growth on C15–C17 (Sorkhoh et al., 1993), *Geobacillus jurassicus* grows only on C6–C16 (Nazina et al., 2005), and *Bacillus thermoleovorans* degrades *n*-alkanes up to C23 (Kato et al., 2001). Indeed, few strains capable of degrading a wide range of hydrocarbons have been identified to date. Some exceptions include *Acinetobacter* sp., M1, which was reported to degrade long-chain *n*-alkanes (C13–C44) (Sakai et al., 1994), and *Rhodococcus* strains capable of degrading *n*-alkanes up to C36 (van Beilen et al., 2003). Degradation of a wide range of hydrocarbons and crude oil is of crucial importance for bioremediation of oil contamination and microbial enhanced oil recovery (Atlas and Atlas, 1991). Unfortunately, relatively few bacteria strains have been isolated and studied.

Here, a novel *Dietzia* strain is reported for the first time that was capable of utilizing a wide range of hydrocarbons (C6–C40), aromatic compounds, and crude oil with different patterns over time.

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2. Methods

2.1. Isolation and identification of strain DQ12-45-1b

Bacterial strains were isolated from an oil production water sample collected from a well-head, in a deep subterranean oil-reservoir in Daqing Oilfield, northeastern China, by cultivation on agar plates composed of 10 g of peptone, 5 g of yeast extract, 10 g of NaCl and 15 g of agar in 1000 ml of oil-production water with the pH of 7.2–7.6 (Medium 1) at 30 °C for 7 days. Selection of strains able to degrade hydrocarbons was conducted by cultivation on agar plates composed of (l^{-1} H_2O) NaCl, 5 g; $NH_4H_2PO_4$, 1 g; $(NH_4)_2SO_4$, 1 g; K_2HPO_4, 1 g; $MgSO_4$, 0.2 g; KNO_3, 3 g with the pH of 7.2–7.6 (Medium 2) and amended with 10 g crude oil for 7 days at 30 °C. One strain, designated strain DQ12-45-1b, was selected due to its good ability of alkane degradation and to its growth assessed at different temperatures (4–45 °C), pH (6.0–12.0), and salinities (1–20% NaCl, w/v). The morphological, physiological and phylogenetic characteristics of the strain were analyzed according to procedures that have been described previously (Wang et al., 2007).

2.2. Utilization of n-alkanes and aromatic compounds as sole carbon sources by strain DQ12-45-1b

To test the ability of strain DQ12-45-1b to grow on different hydrocarbons as sole carbon sources, cells from 5 ml 3-day-old preculture (10^9 CFU ml^{-1}, CFU: colony forming unit) in Luria–Bertani (LB) medium were harvested by centrifugation at 8000 rpm for 5 min and then washed twice with sterile saline solution to exclude the residual organic compounds from the preculture solution, after which they were inoculated into 100 ml of Medium 2 supplemented with liquid or solid *n*-alkanes in 250 ml Erlenmeyer flasks. The final concentrations of *n*-alkanes (C6, C8, C10, C12, C14, C16, C20, C24, C28, C32, C36 and C40), cyclohexane (C6), a branched alkane 2,2,4,4,6,8,8-heptamethylnonane (HMN, C16), and aromatic naphthalin, fluorene, phenanthrene, and chrysene in the flasks were 0.3% (v/v) for liquid alkanes, 0.05% (w/v) for solid alkanes and aromatic hydrocarbons. Two controls, one containing cells and no hydrocarbons and another containing hydrocarbons and no cells, were used for calculation of the background baseline of growth and degradation. All cultures were incubated in the dark at 30 °C while shaking at 150 rpm. In the case of *n*-alkanes C14, C16, C20, C24, C28, C32, C36 and C40, five parallel flasks were incubated. The purity of all hydrocarbons used in this study was over 99%, and have been checked by instrumental analysis with the methods described below.

At day 7, 14, and 21, flasks each containing C14, C16, C20, C24, C28, C32, C36 and C40 alkanes were sacrificed to analyze the residual *n*-alkanes by Gas Chromatography (GC, Shimadzu, Kyoto, Japan) respectively. In addition, the cell numbers (based on CFUs) were determined by plating aliquots of the samples on LB agar plates that were incubated at 30 °C for 2–3 days. Moreover, the mixtures in all remaining flasks were sampled every 2 days and analyzed for cell growth based on CFU counts. For other carbon sources, only analyze the cell growth based on CFU counts but no residual quantity analysis.

2.3. Degradation of crude oil by strain DQ12-45-1b

After growth in LB medium at 30 °C for 3 days, cells in 5 ml broth (10^9 CFU ml^{-1}) were harvested by centrifugation at 8000 rpm for 5 min, washed twice with distilled saline solution and inoculated into Medium 2 (100 ml) in 250 ml Erlenmeyer flasks amended with 5% (v/v) crude oil from Oil Product No. 3, Daqing Oilfield (containing: saturated hydrocarbons, 64.08%; aromatic hydrocarbons, 18.91%; asphaltine, 1.61% and non-hydrocarbons, 15.40%). The culture was then incubated in the dark at 30 °C while shaking at 150 rpm. Mixtures in the flasks were sampled to analyze the turbidity (LP2000 Turbidity Meter, HANNA Instruments, Italy), interface tension (Spinning Drop Interfacial Tensiometer, TX500-C, TX500, USA) and residual hydrocarbons.

2.4. Analyses of hydrocarbons

The residual *n*-alkanes in the 100 ml cultures from the flasks containing *n*-alkanes C14, C16, C20, C24, C28, C32, C36 and C40 were extracted from the culture with 100 ml *n*-hexane in a separating funnel. The extracted alkanes were then adjusted to a final volume of 100 ml with *n*-hexane. The concentrations of the extracted alkanes were analyzed using a GC (Shimadzu, Kyoto, Japan) equipped with an on-column injector, FID detector, and a Rtx-1 capillary column (30 m × 0.25 mm i.d., 0.25um thickness). Nitrogen was applied as a carrier gas at a flow rate of 35 ml min^{-1} for *n*-alkane C14, C16 and C20, 21 ml min^{-1} for C24 and C40, and 45 ml min^{-1} for C28, C32 and C36, which was used as an improved method of references (Wang et al., 2006). The oven programs were as follows: initially set to 140 °C for 1 min, followed by increasing to 280 °C at 20 °C min^{-1} for C14, C16 and C20; initially at 155 °C for 2 min and then increasing to 280 °C at 25 °C min^{-1} for C24 and C40; initially set at 240 °C for 1 min and then increasing to 300 °C at 20 °C min^{-1} for C28, C32 and C36. The injector and detector temperatures were 260 and 280 °C for C16, and 300 and 350 °C for C24, C28, C32, C36 and C40, respectively. The extraction recovery rate was calculated by comparing the concentrations of an alkane before and after extraction, which were 95.10 ± 2.57% for C14, 92.16 ± 3.43% for C16, 91.2 ± 3.68% for C20, 85.70 ± 4.12% for C24, 98.48 ± 1.34% for C28, 88.22 ± 5.17% for C32, 92.52 ± 2.75% for C36, and 79.20 ± 4.59% for C40. These values were then used to calculate and normalize the degradation rates of different alkanes.

Residual oil in crude oil cultures was extracted as previously described (Wang et al., 2006). Briefly, 100 ml of the mixture were extracted twice with 100 ml *n*-hexane, after which the oil was collected by centrifugation at 14,000 rpm for 10 min to remove water from the mixture and then air-dried in a fume hood overnight. The crude oil extract was then analyzed by a capillary gas chromatography (GC, Varian-3700, Varian Company, USA) using a GC equipped with an on-column injector, FID detector, and elastic quartz capillary column (30 m × 0.25 mm i.d., 0.22 μm thickness). Nitrogen was applied as the carrier gas at a flow rate of 30 ml min^{-1}. The oven temperature was set to 50 °C for 1 min, after which it increased to 310 °C at a rate of 6 °C min^{-1}. The injector and detector temperatures were 310 and 320 °C, respectively. Pristane was selected as a common internal standard (Didyk et al., 1978). The relative abundance of different chain-length (C12–C40) hydrocarbons was calculated as the ratio of the peak area of each hydrocarbon to the peak area of pristane in the GC chromatograph.

2.5. Detection of n-alkane degradation genes

In general, the integral-membrane alkane monooxygenase (AlkB) and the cytochrome P450 enzymes (CYP) belonging to CYP153 family are the two types of alkane hydroxylases responsible for the degradation of *n*-alkanes ranging from C5 to C16 in bacteria. The degenerate forward primer (CYP153-F1, 5′-ATGTTYA TYGCNATGGAYCCN) and reverse primer (CYP153-R2 5′-GCGRTTVCCCATRCARCGRTG) were designed based on the conserved domains "MFIAMDP" and "HRCMGNR" of CYP153, respectively, according to the multiple amino acid sequences alignment of CYP153 superfamily genes and used to amplify the

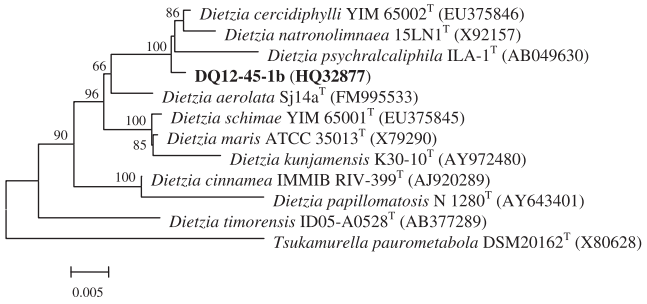

Fig. 1. Phylogenetic relationship based on the 16S rDNA gene sequences between strain DQ12-45-1b and species in the genus *Dietzia* as determined by the neighbor-joining algorithm using the Jukes–Cantor correction factor in the ARB program. Tree topology was also evaluated by the Maximum-Likelihood and Maximum Parsimony algorithms in the ARB program. Bootstrap values over 70% are shown at the nodes. Bar, 2 nucleotide substitutions per 100 nucleotides.

fragment of CYP153 gene(s), an approximate 820 bp PCR product. After grown in LB medium for 3 days, DNA of strain DQ12-45-1b was extracted with the standard method (Marmur, 1961). *alkB* and CYP153 genes were then detected by PCR using the primer pairs: alkB-1f 5′-AAYACNGCNCAYGARCTNGGNCAYAA and alkB-1r 5′-GCRTGRTGRTCNGARTGNCGYTG for *alkB* gene fragment (Kloos et al., 2006) and CYP153-F1 and CYP153-R2 for CYP153 gene, respectively and the following program was used: 5 min at 95 °C; 35 cycles of 45 s at 94 °C, 30 s at 54 °C and 60 s at 72 °C; 10 min at 72 °C; and indefinitely at 10 °C, The PCR products were detected by electrophoresis in the 1.5% agarose gel.

Total RNA was also extracted from the collected cells grown on different *n*-alkanes using Trizol Reagent (Invitrogen, Carlsbad, California, USA), treated with DNase I and purified by Trizol Reagent. Reverse transcription was performed on 0.5 g total RNA with random primers and the ReverTra Ace reverse transcription kit (TOYOBO, Japan). The 16S rRNA gene was utilized as the internal control to normalize the total RNA integrity. Gene specific primers were designed, including alkBrtF (ttcctgtcgatgccgttcgt) and alkBrtR (tcgagcttgtccatcacctcgt), CYPrtF (ccgaatcgccaagactgacact) and CYPrtR (tacgccgaacccgaagga), 16rtF (gtctcatgttgccagcacgtt) and 16rtR (gcagccctctgtactagccat), for amplifying *alkB*, CYP153 and 16S rRNA genes respectively. PCR for *alkB*, CYP153 and 16S rRNA genes was performed for 2 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C.

3. Results and discussion

3.1. Isolation and description of Dietzia strain DQ12-45-1b

Strain DQ12-45-1b (deposited in the China General Microbiological Culture Collection Center with the deposit number of CGMCC 1.10709) was one of the 140 strains isolated from the

Table 1
Comparisons among the ranges of carbon sources utilized by different *Dietzia* strains.

Carbon source	Growth				
	DQ12-45-1b[a]	Strain A14101[b]	*D. maris* DSM43672[TB]	*D. maris*[c]	*Dietzia* sp. E1[d]
n-C5		–	–		
n-C6	+	+		+	+
n-C7		+		+	
n-C8	+	+		+	
n-C9		+		+	
n-C10	+	+		+	+
n-C11		+		+	
n-C12	+	+		+	+
n-C13				+	
n-C14	+	+		+	
n-C15				+	
n-C16	+	+		+	+
n-C17		+	+	+	
n-C18		+	+		+
n-C19				+	
n-C20	+				+
n-C22					+
n-C23				+	
n-C24	+				
n-C28	+				
n-C30					+
n-C32	+				
n-C36	+				
n-C40	+				
Cyclohexane (C6)	+				
Pristane		+	+		+
Naphthalin	+				
Fluorene	+				
Phenanthrene	+				
Chrysene	+				
HMN (C16)	+				
Toluene	–	–	–		
Benzene	–	–	–		
Ethylbenzene	–	–	–		
o-Xylene	–	–	–		

Empty spaces means not determined, (+) means obvious growth, (–) means no growth.
[a] This study.
[b] Data from Bødtker et al. (2009).
[c] Data from Duckworth et al. (1998) and Nesterenko et al. (1982).
[d] Data from Bihari et al. (2010).

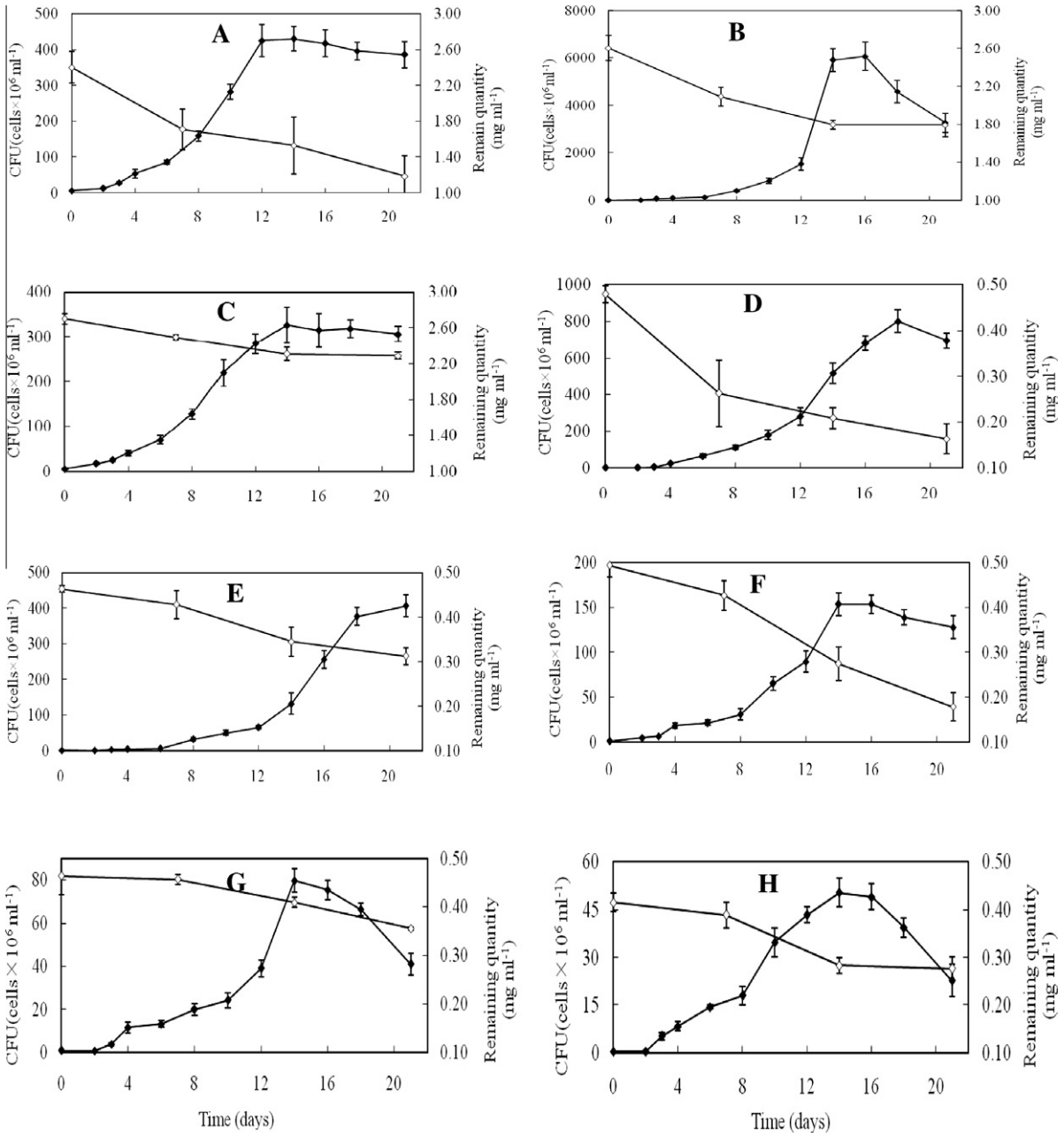

Fig. 2. Growth of *Dietzia* sp. DQ12-45-1b on different *n*-alkanes as the sole carbon sources (indicated as CFU count in the left vertical axis) and its degradation of the the alkanes (indicated as decrease of residual *n*-alkanes in right vertical axis). A, B, C, D, E, F, G and H represented the case of *n*-alkane C14, C16, C20, C24, C28, C32, C36 and C40, respectively.

production water collected from a well-head, in a deep subterranean oil-reservoir from No. 3 Oil-Product, Daqing Oilfield. The nearly-complete 16S rRNA gene sequence (1441 bp) of strain DQ12-45-1b (with the GenBank accession number being HQ32877) was obtained and phylogenetic analysis suggested that it belonged to *Actinobacteria*, forming a stable clade with the type strains of all species in the genus *Dietzia* and showing the highest 16S rRNA sequence similarity of 99.358% with *Dietzia cercidiphylli* (Fig. 1). The microorganism was Gram-positive, facultatively anaerobic, non-endospore-forming, oval to rod-shaped (0.48 × 0.5–1.0 μm), non-motile with no flagellum. Colonies (0.5–1 mm) grown on LB agar for 3 days at 30 °C were smooth, circular convex, wet and orange in color. The organism grew at 4–45 °C, pH 6.0–12.0 and 0–20% (w/v) NaCl, with the optimum growth occurring at pH 8.0, 30 °C and 2% (w/v) NaCl. The strain was positive for catalase and urease, and negative for oxidase, aerobic nitrite reduction, anaerobic nitrate reduction and denitrification, as well as for the hydrolysis of Tween 80, starch and gelatin. Morphological, physiological and phylogenetic properties suggested that strain DQ12-45-1b was a member of the genus *Dietzia*.

3.2. Utilization of n-alkanes (C6–C40) and other compounds as the sole carbon sources by Dietzia sp. DQ12-45-1b

When *n*-alkanes (C6–C40) and other compounds were tested as the sole carbon sources for strain DQ12-45-1b, growth was observed in all cases. The strain grew obviously and rapidly with *n*-alkanes including C14, C16, C20, C24, C28 and C32, while it grew a bit slower when *n*-alkanes including C6, C8, C10, C12, C36, C40, cyclohexane (C6), HMN (C16), and aromatic hydrocarbons

Table 2
Growth of strain DQ12-45-1b with diferent hydrocarbons as the sole carbon sources.

Carbon source	Lag phase (days)	Duplication time (hours)[a]	Yield	
			Maximum (CFU × 10^6 ml^{-1})	Time (days)
n-C6	3	111.50	44.33	14
n-C8	2	78.21	64	10
n-C10	2	110.09	122.33	14
n-C12	1	106.45	132	12
n-C14	2	56.66	429.33	14
n-C16	2	37.56	6066.67	16
n-C20	2	67.15	325.67	14
n-C24	3	50.434	798.33	18
n-C28	6	100.15	683.33	34
n-C32	3	67.62	153.67	16
n-C36	4	86.73	79.67	14
n-C40	4	92.49	50.33	14
Cyclohexane (C6)	4	62.53	10.07	14
Naphthalin	3	111.63	62	8
Fluorene	2	82.04	75.33	8
Phenanthrene	2	94.12	50.67	8
Chrysene	2	80.27	97.67	14
HMN (C16)	1	66.56	43.33	8

[a] Duplication time was calculated by the formula:

$$N = N_0 \times 2^n \quad (1)$$

$$\lg N = \lg N_0 + n\lg 2 \quad (2)$$

$$n = 3.322(\lg N - \lg N_0) \quad (3)$$

$$G = \frac{t2 - t1}{3.322(\lg N - \lg N_0)} \quad (4)$$

N: maximum CFU of the exponential phase; N_0: minimum CFU of the exponential phase; n: number of generation; $t2$: time of maximum CFU; $t1$: time of minimum CFU; G: duplication time/generation time.

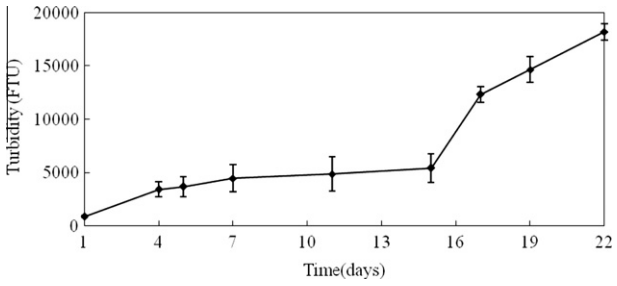

Fig. 3. Turbidity change in the *Dietzia* sp. DQ12-45-1b culture with crude oil.

including naphthalin, fluorene, phenanthrene and chrysene, but could not use benzene, toluene, ethylbenzene and o-xylene for growth. Comparisons with other *Dietzia* strains suggested that strain DQ12-45-1b could use a broader range crude oil components as the sole carbon sources, including aliphatic hydrocarbons, branched alkane, cyclane and aromatic hydrocarbons (Table 1).

Among the C14–C40 *n*-alkanes, CFU counts increased during the first 15–17 days after a 3–7 days lag phase, except for the cases of C24 and C28 (Fig. 2). A remarkable increase in hydrocarbon degradation (the loss of vaporization and extraction was excluded by comparisons with the two blanket controls) was also observed in this period in most cases. However, the maximum CFU counts decreased as the chain-length increased. Specifically, when hexadecane (C14) was used as the sole carbon source, it was degraded by 121.41 mg in the 21-day experiment by a culture unit (100 ml). Comparatively, only 81.17, 40.85, 31.57, 15.06, 31.54, 10.75 and 13.87 mg of C16, C20, C24, C28, C32, C36 and C40 were degraded, respectively, (Fig. 2). Although the degradation of *n*-alkanes did not

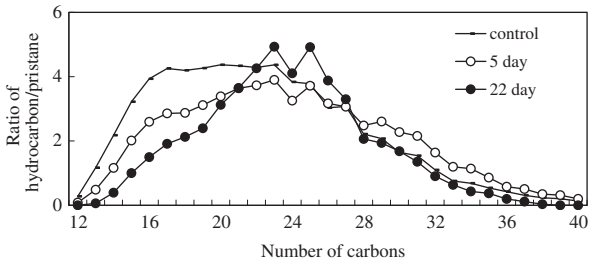

Fig. 4. Relative ratios of different chain-length hydrocarbons to pristane in crude oil. The control represented the initial crude oil without any bacterium addition. The "5 day" and "22 day" stood for the crude oil sampled on the 5th day and the 22nd day.

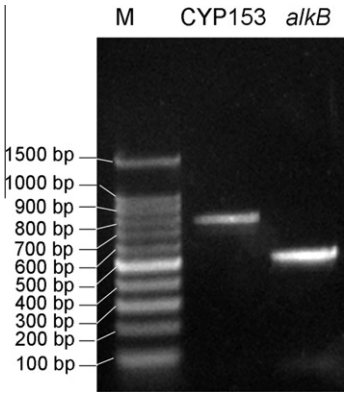

Fig. 5. Detection of *alkB* and CYP153 genes of the strain DQ12-45-1b. Left lane: 100 bp ladder; Middle lane: CYP153; right lane: *alkB*. Both *alkB* and CYP153 genes were amplified with the correct length positive bands in the gel.

show significant difference, the bacterial populations (CFU counts), the lag phase and the duplication times were significantly different among the hydrocarbons (Table 2). Hexadecane might be the best carbon source for the strain to grow while the longer or shorter chain-length alkanes could maintain less or more difficult growth (Table 2).

3.3. Degradation of crude oil by the Dietzia sp. DQ12-45-1b

After strain DQ12-45-1b was inoculated into typical crude oil from Daqing Oilfield, the culture started to become turbid and black at day 4. Specifically, the turbidity (indicated as the Formazan Turbidity Unit, FTU) of the culture solution increased slightly during the first 15 days, after which it increased sharply until the end of the experiment (22 days) (Fig. 3). Additionally, the interface tension (IFT) of the mixture decreased to 6.829 mN m^{-1} at day 22 from 21.414 mN m^{-1} at day 0. After 5 days of cultivation, the hydrocarbons with a chain-length of $\leqslant$25 were degraded, resulting in the ratios of hydrocarbons $\leqslant$C25 to pristane in the oil mixture being smaller than those in the control crude oil. As a result, the relative abundance of hydrocarbons ($\geqslant$C28) to pristane increased and became greater than those in the control crude oil. Interestingly, at day 22, the ratios of hydrocarbons with carbon numbers $\leqslant$C22 and $\geqslant$C28 to pristane both decreased, and this decrease was accompanied by the accumulation of hydrocarbons with carbon numbers ranging from C23 to C27 (Fig. 4).

The diagnostic parameters C21 + C22/C28 + C29 also supported such a two-stage pattern. The C21 + C22/C28 + C29 ratio decreased from 2.01 in the control to 1.45 after 5 days of culture, and increased to 1.97 after 22 days. The widely accepted *n*-alkanes sequential degradation theory suggests that C8 and C12–15 are

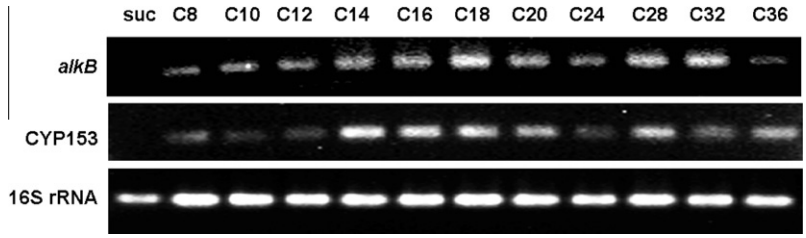

Fig. 6. The induction of *alkB*, CYP153 and 16S rRNA genes by C8–C36 *n*-alkanes. The positive bands in the gel represented the transcripts of mRNAs of *alkB*, CYP153 and 16S rRNA genes.

preferentially degraded during the early stages of crude oil biodegradation (Bødtker et al., 2009), which could be one of the reasons why strain DQ12-45-1b preferentially utilized hydrocarbons ⩽C25 during the first 5 day cultivation (Fig. 3). In the later stage, the degradation of hydrocarbons with carbon numbers from 24 to 28 should be lower that those with the chain length longer than 32 (including C32, C36 and C40) (Fig. 2), leading to the accumulation of C23–C27 hydrocarbons.

Crude oil is a complex mixture primarily composed of insoluble compounds, including different chain-length *n*-alkanes, which are not easily dispersed in water. Production of emulsifying agents or biosurfactants (data not shown) from the degradation of short-chain hydrocarbons by *Dietzia* sp. DQ12-45-1b led to the initial solubility of crude oil and the turbidity of the culture mixture (Fig. 3). Growth of the cells was then enhanced by the 'dissolved' oil, followed by the production of more emulsifying agents. As a result, increasing amounts of oil were dispersed into the culture solution, leading to a sharp increase in the turbidity of the mixture (Fig. 3). It has been reported that different chain-length hydrocarbons are selectively dissolved by certain emulsifying agents (Mulligan et al., 2001); however, further study is needed to determine whether *Dietzia* sp. DQ12-45-1b could produce different emulsifying agents that preferentially dissolve short-chain (⩽C25) and long-chain (⩾C28) alkanes, or if it produces a special emulsifying agent that preferentially dissolves both short-chain (⩽C25) and long-chain (⩾C28) alkanes, thereby leading to the preferential degradation of hydrocarbons ⩽C25 and ⩾C28 (Fig. 4) by AlkB and other alkane hydroxylases.

3.4. Detection of n-alkanes degradation genes

Although microbial degradation of hydrocarbons is reportedly ubiquitous in nature and many short and long chain alkane degrading strains of bacteria have been isolated, few studies have reported microorganisms capable of degrading hydrocarbons from C6 to C40. In general, *n*-alkane degradation occurs via several types of enzymes, among which alkane hydroxylases play an important role. Three classes of alkane hydroxylases have been reported in microorganisms. The soluble non-heme di-iron monooxygenase (sMMO) and membrane-bound particulate copper-containing enzyme (pMMO) primarily catalyze the oxygenation of C1–C5 alkanes (Murrell et al., 2000). In addition, membrane-bound *n*-alkane hydroxylases (AlkB) and some membrane-bound cytochrome P450 enzymes (CYPs, such as CYP153) found in fungi (Maier et al., 2001) and bacteria can oxidize alkanes with chain-lengths ranging from C6 to C16 at the terminal carbon atom (van Beilen and Funhoff, 2005).

The enzyme system that leads to the oxidation of *n*-alkanes with a chain-length longer than C18 is less clear. There are at least two mechanisms involved in the degradation of these compounds. For example, *Pseudomonas fluorescens* CHA0 can utilize a wide range of *n*-alkanes; however, its *alkB* gene knockout mutant, strain KOB2 1, can grow on C18–C32 alkanes, but not on C8–C16 alkanes (Smits et al., 2002). This suggests that an unknown enzyme system other than the AlkB in CHA0 may contribute to the usefulness of *n*-alkanes ranging from C18 to C32. Moreover, although other AlkB homologous enzymes such as AlkMa from *Acinetobacter* sp. M-1 have been shown to be induced by C26 and C30 *n*-alkanes at the mRNA level (Tani et al., 2001), there is still a lack of experimental evidence of its degradation of long-chain alkanes ranging from C18 to C36. Additionally, long-chain alkane monooxygenase (LadA) found in *Geobacillus thermodenitrificans* NG80-2 is the only enzyme that has been found to be capable of hydroxylating *n*-alkanes ranging from C15 to C36 so far (Feng et al., 2007). However, this bacterium could not degrade hydrocarbons with chain lengths smaller than C14. The complete genome sequence revealed that no *alkB* homolog encoding gene was found in NG80-2. LadA is an extracellular protein distinct from AlkB that does not require any coenzymes such as rubredoxin and rebredoxin reductase, which are essential in the AlkB mediated alkane oxidation pathway.

In this study, *LadA* gene was not detected (data not shown). However, two genes encoding alkane monooxygenase (AlkB) and cytochrome P450 alkane hydroxylase of the CYP153 family which belonged to different terminal oxidation pathways in different bacteria and fungi were also amplified from the strain DQ12-45-1b (Fig. 5). In addition, total RNA was isolated from *Dietzia* sp. DQ12-45-1b cells grown on C8–C36 *n*-alkanes and the transcript levels of *alkB* and CYP153 were determined by RT-PCR. The expression of *alkB* and CYP153 transcripts could be induced by C8–C36 *n*-alkanes, peaking at C14–C20 *n*-alkanes (Fig. 6), which was in accordance with the results of growth rates and alkane degradation rates, suggesting that both *alkB* and CYP153 might be related to *n*-alkane degradation of DQ12-45-1b. However, these two genes were reported to be responsible for the degradation of small- and medium-length *n*-alkanes ranging up to C16. In contrast, *Dietzia* sp. DQ12-45-1b was found to utilize *n*-alkanes ranging from C6 to C40 and to show different alkane utilization patterns when growing in crude oil (Fig. 4), suggesting that there might be novel long-chain alkane oxidation pathway(s) other than the AlkB system known in other bacterium. In fact, a novel AlkB rubredoxin fused alkane hydroxylase encoding gene was cloned in strain DQ12-45-1b in an accompanying study with the GenBank Accession Number of HQ850582, which was proved to be responsible for the degradation of longer hydrocarbons. As for the CYP153 gene, it was cloned and sequenced in another accompanying study with the ability to degrade n-alkanes with the chain-length of C14 and C16 (data not shown).

4. Conclusions

A novel *Dietzia* strain was isolated. It was able to grow on C6–C40 alkanes and aromatic compounds as the sole carbon sources. Both *alkB* and CYP153 genes and their induction were detected in the strain and smaller hydrocarbons (⩽C25) in crude oil were preferentially degraded while the medium chain-length hydrocarbons (C23–C27) were accumulated in the later stage of cultivation, indicating the possible different hydrocarbon metabolic pathways in the strain. To date, it is the first *Dietzia* strain reported to have

these functions and can be important for bioremediation of oil polluted environments and microbial enhanced oil recovery.

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