High efficiency degradation of alkanes and crude oil by a salt-tolerant bacterium Dietzia species CN-3

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A bacterial strain, CN-3, was isolated from petroleum-contaminated sediment in Bohai Bay, China. Morphological, physiological and phylogenetic analyses indicated that the strain belonged to the genus Dietzia. It was capable of utilizing a wide range of alkanes (C11–C31), aromatic compounds and crude oil as the sole carbon and energy sources. The bacterium showed high degradation rate (>80%) of the saturated hydrocarbons of crude oil in the pH range of 6–9. The degradation rate was hardly affected at a NaCl concentration up to 85 g L⁻¹, indicating that the strain can tolerate high salinities. The real-time quantitative Polymerase Chain Reaction (PCR) results showed that the CYP153 genes could be significantly induced by C14, C15, C16, C26 and pristane, whereas the transcription level of alkB gene was increased moderately with C14, C15, C16, C26 and pristane induction condition. A high degradation extent, wide substrate range and facile conditions enhanced the potential for this bacterial strain to be used in the bioremediation of crude oil pollution.

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1. Introduction

Crude oil is one of the most important strategic energy sources and chemical industry materials world wide. However, oil spills, as a result of natural or anthropogenic factors, cause dramatic environmental and human health problems (Head et al., 2006; Li et al., 2012). Crude oil is a complex mixture of compounds that can be classified into four main groups: saturated hydrocarbons, aromatic hydrocarbons, resins and asphaltenes (Aylonso-Gutierrez et al., 2011). The search for methods to reduce petroleum pollution is becoming increasingly significant and necessary (Ouyang et al., 2005; Mbadiega et al., 2011).

To date, many technologies have been developed for the treatment of crude oil spills, including sorption (Hussein et al., 2008), thermal treatment (Li et al., 2009), surfactants solutions (Agarwal and Liu, 2015) and so on. Compared with physical and chemical methods, bioremediation — using organisms to remove and degrade crude oil — is recognized as more economical, efficient and environmentally friendly (Margesin et al., 2013; McGinity, 2014; Wang et al., 2011a,b). As degraders, numerous microorganisms that utilize crude oil as their carbon source have been isolated, such as Acinetobacter (Lin et al., 2014), Pseudomonas (Zhang et al., 2011), Rhodococcus (Van Hamme and Ward, 2001) and Alcanivorax (Liu et al., 2010). However, many bacteria can only use a narrow range of substrates. For example, Geobacillus jurassicus grows on C6–C16 alkanes (Naya et al., 2005) and Bacillus thermoleovorans strains utilize alkanaes up to C23 (Kato et al., 2001). Among the bacterial degraders, Dietzia species have more advantages and superiority, which were established in 1995, and 13 species have been reported in recent years (Gharibzahedi et al., 2013). Some Dietzia species described to date have been shown to utilize petroleum hydrocarbons well. Dietzia sp. E1 utilizes C8–C36 alkanes as the carbon source (Bihari et al., 2011) and D. cinnamon strain P4 can consume C11–C36 alkanes (Von der Weid et al., 2007). Moreover, Dietzia strains have the capacity to degrade aromatic hydrocarbons, including naphthalene (Von der Weid et al., 2007), phenanthrene (Al-Awadhi et al., 2007) and fluoranthene (Kumar et al., 2011).

Three classes of alkane hydroxylases, which are related to alkane degradation, have been distinguished in these microbial degraders. Microorganisms degrade short-chain-length alkanes (C1–C4) using methane monoxygenase (MMO) enzymes, which include soluble non-heme di-iron MMO and membrane-bound particulate copper-containing MMO (Murrell et al., 2000; Van Beilen and Funhoff, 2005; Mbadinga et al., 2011).
2. Materials and methods

2.1. Sediment, crude oil and reagents

To isolate and screen crude-oil-degrading bacteria, a sediment sample was collected from the contaminated site in Bohai Bay, China. It was capable of utilizing a wide range of alkanes (C_{14}–C_{31}), aromatic hydrocarbons and crude oil as the sole carbon and energy sources. Transcription level of the alkB and CYP153 alkane degradation genes was detected, explaining the high biodegradation of a wide range of alkanes by Dietzia sp. CN-3.

2.2. Isolation and identification of bacteria

The sediment sample (10 g) and crude oil (0.5 g) were inoculated in 100 mL of Bushnell-Haas Broth (BHB) mineral medium (Sinopharm Chemical Reagent, Beijing, China) and then incubated for 7 d at 30 °C with shaking at 180 rpm. The BHB medium contained (per liter): 1 g K_2HPO_4, 1 g KH_2PO_4, 0.2 g MgSO_4·7H_2O, 0.02 g CaCl_2, 1 g NaH_2PO_4, 0.05 g FeCl_3, and 25 g NaCl, pH 7.0. One milliliter Na_2SiO_3 solution was added to 100 mL of cultures. Aliquots (1.8 mL) of liquid culture were used for further analysis. Then the cultures were harvested by centrifugation for 10 min at 6000 rpm and washed three times with BHB mineral medium to remove the interference of the carbon source in the BHB broth medium. The bacteria were incubated in 0.5% (w/v) crude oil medium for 12 d at 30 °C with shaking at 180 rpm. The bacteria-free control was incubated and analyzed in the same way as the experimental groups. All samples were carried out in triplicate and the results are shown as mean values and standard deviations.

The residual oil in the 100 mL of cultures was extracted using three equal volumes of n-hexane. The extractants were collected, dried in anhydrous sodium sulfate and concentrated using vacuum rotary evaporation (Bost et al., 2001). The concentration of residual oil was determined using an infrared spectrometric oil detector (OIL 460, Beijing China Invent Instrument Tech. Co., Ltd, China) (Lang and Tilotta, 2003). The crude oil components, including saturated hydrocarbons, aromatic hydrocarbons, resins and asphaltene, were separated using the column chromatography method (Head et al., 2006).

The saturated hydrocarbons were analyzed using a 7890A gas chromatography–mass spectrometer (GC–MS) (Agilent, Santa Clara, CA, USA) with a capillary column (HP-5 model, 30 m × 0.32 mm × 0.25 μm). The operating conditions were as follows: nitrogen was used as the carrier gas with a flow rate of 2 mL min^{-1}; the injector and detector temperatures were 280 °C and 300 °C, respectively; the column temperature was kept at 80 °C for 2 min and then raised to 280 °C at a rate of 5 °C min^{-1}, which was maintained for 20 min.

Table 1

| Primer       | Sequence (5'–3')              | Reference                  |
|--------------|-------------------------------|----------------------------|
| 27F          | AAGTTTGTACATGGCTCAG           | (Lin et al., 2014)         |
| 1492R        | CCGYTACCTGTGTTAGACATT         |                           |
| CYP_EF       | CATGCTTCTAGTCTACG             | (Wang et al., 2011a,b)     |
| CYP_ER       | CCGAAGCTTGGTTCGACTG           |                           |
| ALKB_F       | GCACCAAGCGAATGCACTACGCAG     | This study                 |
| ALKB_R       | GCACCAAGCGAATGCACTACGCAG     | This study                 |
| alkB-F       | GCCAAATACCGCGTCCTCCAG         | This study                 |
| alkB-R       | CGACTCCTACCGCTTGCCCTGC       | This study                 |
| CYP-F        | GGTCGATCTGCTCG                | This study                 |
| CYP-R        | ATGCGCCGACGTTGCTG            | This study                 |
| 16S-F        | GTCCGCTGCTGGAAATGCTCGG        | This study                 |
| 16S-R        | TCACGGCGTACATGCTACGCGCGCG    | This study                 |
2.4. Effect of pH and sodium chloride concentration on crude oil biodegradation

Strain CN-3 was cultivated in 2216E broth medium at 180 rpm, 30 °C for 48 h, \( (2.15 \times 10^9 \text{ CFU mL}^{-1}) \). The bacterial cells were collected by centrifugation (6000 rpm for 5 min) and re-suspended in BHB mineral medium. The effect of pH on crude oil biodegradation was evaluated by modifying the pH to 5, 6, 7, 8, 9 and 10, individually, using either 1 mol L\(^{-1}\) NaOH or 1 mol L\(^{-1}\) HCl solution.

The influence of NaCl concentration on crude oil biodegradation was investigated using a series of NaCl concentrations: 10, 25, 40, 55, 70 and 85 g L\(^{-1}\). After 12 d cultivation, crude oil was treated as described in section 2.3, and the saturated hydrocarbons biodegradation efficiency in crude oil was measured using GC–MS. The GC–MS conditions were as described above. A control containing all of the materials without an inoculum was used to detect any abiotic loss of saturated hydrocarbons. Each experiment was performed in triplicate and the results are shown as mean values and standard deviations.

2.5. Utilization of alkanes and aromatic compounds by strain CN-3

To test the ability of strain CN-3 to grow on different hydrocarbons as the sole carbon source, the culture was cultivated in 2216E broth at 180 rpm, 30 °C for 48 h, \( (2.15 \times 10^9 \text{ CFU mL}^{-1}) \). The bacterial cells were collected by centrifugation at 6000 rpm for 5 min and then re-suspended in BHB mineral medium. The substrates included \( n \)-alkanes (C\(_{14}, C_{15}, C_{16}, C_{26}\)) , a branched alkane (pristane) and aromatic hydrocarbons (phenanthrene and pyrene). The original concentrations in the flasks were 1000 mg L\(^{-1}\) for the alkanes and 400 mg L\(^{-1}\) for the aromatic hydrocarbons. The controls contained hydrocarbons but no cells, and were used to calculate the background values of growth and degradation. All cultures were incubated at 30 °C with shaking at 180 rpm. After 12 d cultivation, the culture medium was extracted three times with an equal volume of \( n \)-hexane, dried over anhydrous sodium sulfate and evaporated to dryness. Samples containing the

![Fig. 1. Neighbor-joining algorithm trees derived from gene sequences detected in Dietzia sp. CN-3. Trees were constructed using MEGA 6.0. Bootstrap values shown at the nodes for frequencies at or above a 60% threshold (1000 bootstrap resampling). Phylogenies based on the 16S rRNA gene sequence from other Dietzia strains. Tsukamurella paurometabola DSM 20162 was used as outgroup. Bar indicates 0.005 substitutions per nucleotide position.](image1)

![Fig. 2. Mass chromatogram of biodegradation efficiency of saturated hydrocarbons in crude oil by Dietzia sp. CN-3 in 0 day (A) and 12 day (B) cultivation.](image2)

| Alkane | Degradation (%)\(^a\) | Alkane | Degradation (%)\(^a\) |
|--------|----------------------|--------|----------------------|
| C\(_{16}\) | 96.94 | C\(_{25}\) | 95.23 |
| C\(_{17}\) | 94.5 | C\(_{26}\) | 98.48 |
| C\(_{18}\) | 94.83 | C\(_{27}\) | 96.06 |
| C\(_{19}\) | 98.08 | C\(_{28}\) | 90.7 |
| C\(_{20}\) | 99.2 | C\(_{29}\) | 94.03 |
| C\(_{21}\) | 98.94 | C\(_{30}\) | 81.28 |
| C\(_{22}\) | 98.93 | C\(_{31}\) | 84.21 |
| C\(_{23}\) | 95.75 | Pristane | 93.4 |
| C\(_{24}\) | 97.85 | Phytane | 87.72 |

\(^a\) All results of biodegradation were calculated as the difference between the treatment and the control groups. The treatment groups contain bacteria and crude oil in the medium, and the control groups had corresponding crude oil without bacteria in the medium.
above-mentioned substrates were analyzed using a GC–MS (Agilent 7890A) with a capillary column (DB-5 model, 30 m x 0.25 mm x 0.25 μm). The operating conditions were as follows: helium was used as the carrier gas with a flow rate of 2 mL min⁻¹; the injector and detector temperatures were 280 °C and 300 °C, respectively; the column temperature was 50 °C for 2 min, with a ramp to 100 °C at a rate of 5 °C min⁻¹, raised to 200 °C at a rate of 25 °C min⁻¹, and then raised to 260 °C at a rate of 15 °C min⁻¹, which was maintained for 10 min.

To assess the degradation efficiency, control experiments that had corresponding substrates without bacteria were tested, and the remaining hydrocarbon concentrations in the cultures were calculated according to the standard curve. The n-octadecane (20 mg L⁻¹) was used as internal standard to calibrate in GC-MS measurement. All experiments were in triplicate and the results are expressed as mean values and standard deviations.

2.6. Detection of alkane degradation genes by real-time quantitative PCR

The alkane degradation genes were amplified and detected to reveal the degradation activity and mechanism. The alkB and CYP153 genes were amplified with the primer pairs CYP_EF and CYP_ER (Wang et al., 2011a,b), and ALKB_F and ALKB_R (Table 1), generating approximately 1365 bp and 1485 bp PCR products, respectively. After growth in 2216E medium for 3 d, total genomic DNA of strain CN-3 was extracted using the method described above. The transcription of CYP153 and alkB genes was then detected by PCR using the above primer pairs and the following program: 94 °C for 10 min; 30 cycles of 30 s at 94 °C, 30 s at 55 °C and 1 min at 72 °C, and indefinitely at 12 °C. Then the PCR products were checked by electrophoresis in 1% agarose gel.

Total RNA was extracted from the bacterial cultures cultivated on C14, C15, C16, C26 and pristane using the hot phenol method (Min et al., 2016). The quantity and quality of the RNA were confirmed by spectrophotometry (Nanodrop, 2000 °C, Thermo Scientific, Waltham, MA, USA). Reverse transcription was performed on 3 μg of total RNA with random primers in a final volume of 20 mL using the Transcript One-Step gDNA Removal and cDNA Synthesis SuperMix kit (Transgen Biotech, Beijing, China).

For real-time quantitative PCR (RT-qPCR), 0.2 μL of cDNA was mixed with 2 × Trans Start Tip Top Green qPCR Super Mix (TransGen Biotech), 0.2 μmol of each primer and 0.4 μL passive reference dye in a final volume of 20 μL in three replicates. No-template controls were included. The 16s rRNA gene was used as the internal control to normalize the expression levels of investigated genes. Gene specific primers, including alkB-F and alkB-R,
CYP-F and CYP-R, and 16S-F and 16S-R, were designed to amplify the alkB, CYP153 and 16S rRNA genes, respectively. Real-time qPCR was carried out using the ABI Prism 7500 Fast Sequence Detection System (Applied Biosystems, Foster City, CA, USA) with the following protocol: 95 °C for 15 min; 40 cycles of 30 s at 95 °C, 30 s at 61 °C and 30 s at 72 °C. The normalized relative transcript levels were determined using the 2−ΔΔCt method (Livak and Schmittgen, 2001).

3. Results and discussion

3.1. Isolation and description of Dietzia sp. CN-3

Strain CN-3 (deposited in the China General Microbiological Culture Collection Center with deposit number CCTCC M 2015537) was one of the five strains isolated from petroleum-contaminated sediment in Bohai Bay, China. Compared with other strains, CN-3 showed dramatic crude oil degradation efficiency through its biomass growth trend (Colony Forming Units counts), as well as the GC–MS measurements.

After 3 d incubation in 2216E plates, strain CN-3 formed circular, smooth, wet, orange colonies (0.5–1.0 mm). Gram staining showed that strain CN-3 was Gram-positive. Scanning electron microscopy revealed that it was a rod-shaped (0.5 x 1.0 μm) bacterium. The 16S rRNA gene sequence of strain CN-3 (GenBank accession number KT779094) was obtained. The 16S rRNA gene sequence comparisons indicated that strain CN-3 shows a high similarity (99%) to the previously isolated bacterium Dietzia sp. ZQ-4, which belongs to the phylum Actinobacteria. Based on the phenotypic characteristics and phylogenetic analysis, strain CN-3 was determined to be a member of the genus Dietzia (Fig. 1).

3.2. Dietzia sp. CN-3 crude oil biodegradation efficiency

Crude oil is a complex mixture of thousands of individual compounds (Head et al., 2006). To examine the crude oil degradation activity of Dietzia sp. CN-3, cells was cultivated in BHB medium containing 0.5% (w/v) crude oil as the sole carbon and energy source. Infrared spectroscopy was used to determine the residual crude oil after biodegradation by Dietzia sp. CN-3. After 12 days, we found the crude oil was dispersed into small droplets and the medium became turbid and black in the flasks containing 0.5% (w/v) crude oil and bacteria. The residual crude oil clearly decreased during the experiment process, about 33.5% of the crude oil was degraded after 12 d, with a concentration changed from 4970 to 3310 mg L−1. The degradation rate varied slightly from day 8 to day 12. In contrast, the control group with no bacteria showed no crude oil degradation.

Furthermore, as the mass chromatogram demonstrated (Fig. 2), a notable decrease in saturated hydrocarbons observed crude oil degradation microorganisms to degrading. The GC–MS analysis results showed that strain CN-3 was able to degrade a wide range of alkanes (C16–C31) in crude oil, plus pristane and phytane (Table 2). About 68.4% of saturated hydrocarbons were utilized in the first 2d and 19.7% in the following 2d, and the degradation rate was progressively slower in the following days (Fig. 3). This might be attributed to the biomass of the bacterium and the absorption of crude oil, which means the bacteria might have reached the peak degradation activity in the first four days (Zhang et al., 2011). After 12d, the degradation rate was 91.6%, which is higher than previously reported rates (66.6–82.5%), even for mixed bacterial consortia (Gao et al., 2015; Von der Weid et al., 2007). In the aspect of practicability for clean-up processes, degradation of a wide range of hydrocarbons is significant for bioremediation of oil contamination (Atlas and Atlas, 1991). For example, in a field-scale study, 13–23% total hydrocarbon content was degraded by oil degradation microorganisms in the oily sludge and soil after 56 days (Ouyang et al., 2005). In another study, the bacterial strains obtained from oil reservoirs were evaluated for petroleum degradation ability. In particular, the Dietzia maris CBM47 705 showed high degradation rates (>90%) against the saturated hydrocarbons (Dellagnezze et al., 2014).

Owing to the complex composition of crude oil, many bacterial strains could only utilize a narrow substrate ranges of crude oil fractions or have low degradation efficiency. The superiority of Dietzia sp. CN-3 was that it could utilize crude oil as the sole carbon and energy source and it showed high degradation efficiency on a wide range of alkane compounds (C16–C31), which may be of great significance in bioremediation of oil-contaminated soil and wastewater.

3.3. Effect of pH and sodium chloride concentration on crude oil biodegradation

The optimum pH level for crude oil degradation by Dietzia sp. CN-3 was investigated at 30 °C in a rotary shaker (180 rpm). In the pH range of 5–10, the degradation rate of the saturated hydrocarbons clearly changed, varying from 47.4 to 92.7% (Fig. 4A) at pH 5 and 7, respectively. The degradation rate was approximately the same in the pH range of 6–9. However, the degradation rate decreased drastically by about 24% when the pH was adjusted from pH 9 to 10, which means that the degradation activity of the bacterium was inhibited at this point. Dietzia sp. CN-3 showed better activity for degradation of the saturated hydrocarbons in crude oil in neutral and weak alkaline conditions. Overall, Dietzia sp. CN-3 was able to grow at pH between 5 and 10, with an optimal pH of 7.0, at which Dietzia has the highest enzymatic activity for degradation of the saturated hydrocarbons in crude oil (Duckworth et al., 1998; Wang et al., 2007; Yumoto et al., 2002).

To investigate the salinity tolerance of Dietzia sp. CN-3 and crude oil degradation activity of this strain at different levels of sodium chloride concentration, the crude oil degradation experiments were carried out at NaCl concentrations ranging from 10 to 85 g L−1 at pH 7.0. The degradation rate of the saturated hydrocarbons decreased slowly with the increasing of NaCl concentrations, varying from 85.6 to 91.9% (Fig. 4B). Dietzia sp. CN-3 had the highest activity at the NaCl concentration range from 10 to 25 g L−1. The growth curve of Dietzia sp. CN-3 at different NaCl concentrations revealed that salinity had little effect on the bacterial biomass, and in general, biomass is strongly related to the degradation rate (Wang et al., 2007). In addition, the degradation rate was still high (>85%) at the NaCl concentration of 85 g L−1, demonstrating that high salinity does not inhibit the degradation activity of the bacteria. The results indicated that the concentration of NaCl had a
limited impact on the degradation of saturated hydrocarbons in crude oil. Dietzia sp. CN-3 had a high salinity tolerance and showed a high rate of saturated hydrocarbons degradation at elevated salinities (10–85 g L−1). So Dietzia sp. CN-3 could also utilize a wide range of alkanes (C14–C17), aromatic compounds and crude oil as the sole carbon and energy sources, which could prove beneficial guidance for the bioremediation of crude oil pollution.

3.4. Degradation of alkane and aromatic hydrocarbon compounds by Dietzia sp. CN-3

The effective degradation of crude oil was measured as above; therefore, the degradation of pure alkanes and aromatic hydrocarbons were tested as the sole carbon and energy source for Dietzia sp. CN-3 (Fig. 5). In the experiment process, Dietzia sp. CN-3 grew rapidly in BH medium supplemented with n-alkanes C14, C15 and C16, while it grew slightly slower in medium containing C26-pristane, phenanthrene and pyrene.

Generally, alkanes are vulnerable to microbial attack (Lin et al., 2014). Therefore, C14, C15 and C16 were found to be significantly degraded by Dietzia sp. CN-3. Because of the hydrophobicity and higher molecular weight of longer-chain alkanes, the degradation rate (54.2%) of C26 was much lower than the middle-chain n-alkanes (C14, C15, and C16). As an isoaromatic alkane, pristane is one of the most widely used biomarkers (Rontani and Bonin, 2011), and it is difficult to degrade by some bacteria (Dawson et al., 2013; Rontani and Bonin, 2011). For example, Dietzia sp. P4, has a high affinity for C1–C36 alkanes (52.4–88.11%), but a low affinity for pristane (45.41%) (Von der Weid et al., 2007). However, according to the bacterial growth and GC–MS results in this experiment, Dietzia sp. CN-3 could degrade pristane up to 74%.

Polycyclic aromatic hydrocarbons (PAHs) are another group of compounds that are ubiquitously dispersed in crude oil. Owing to their toxicity and carcinogenic potency, PAHs are resistant to biodegradation (Wu et al., 2013). In this study, degradation of three- and four-ring PAHs by Dietzia sp. CN-3 was investigated. As shown in Fig. 5, 15.2% and 14.6% of phenanthrene and pyrene, respectively, were degraded after 12d. Degradation of PAHs by bacteria has been broadly studied all over the world (Wu et al., 2010). Dietzia species have been reported to degrade PAHs, such as Dietzia sp. DQ12-45-1b, which can grow in medium containing naphtalin, fluorene and phenanthrene (Wang et al., 2011a,b).

3.5. Detection of alkane degradation genes

Alkane hydroxylation is the first and key step in alkane degradation. The process needs several classes of enzymes, among which alkane hydroxylases play a crucial role (Van Beilen and Funhoff, 2005). The alkB and CYP153 genes are medium-chain-length alkane degradation genes that have been found in many bacteria (Kato et al., 2001; Li et al., 2008). However, the enzyme systems resulting in the oxidation of long-chain-length alkanes (>C18) are relatively unclear, and they may be unrelated to the above alkane hydroxylase genes. The almA gene, encoding a flavin-binding monooxygenase, was found in the Acinetobacter sp. DSM 17874. It was confirmed by experiments that the almA gene was related to the oxidation of long-chain-length alkanes of C12 and longer (Throne-Holst et al., 2007). In addition, almA gene sequences were found in several other strains, including Alcanivorax, Acinetobacter, Marinobacter and Parvibaculum (Wang and Shao, 2012). The LdA gene, found in Geobacillus thermotolerans NC80-2, oxidizes C14–C36 alkanes, but does not utilize chain lengths smaller than C14 (Feng et al., 2007). The complete genome sequence indicated that there is no alkB homolog encoding gene in NG80-2.

In this study, the LdA and almA genes were not detected to date. However, two genes, CYP153 and alkB, used to encode soluble cytochrome P450 alkane hydroxylase and alkane monooxygenase were cloned, respectively. In general, they are frequently associated with the degradation of small- and medium-length alkanes (C5–C17) (Rojo, 2009). Phylogenetic analysis of both the alkB and CYP153 coded genes isolated from Dietzia sp. CN-3 revealed the enzymatic versatility of the genus (Fig. 6A and B). The enzymes coding by alkB and CYP153 genes, which are found with high diversity among Gram-positive bacteria, are structurally distant enzymes (Van Beilen and Funhoff, 2005). The functional sense of different alkane hydroxylase systems is unknown and could be concerned with the oxidation of a different range of alkanes. This would be worthwhile studying in the future.

Total RNA was extracted from Dietzia sp. CN-3 cells grown on different n-alkanes (C14, C15, C16 and C18) and a branched alkane (pristane), and the transcript levels of the alkB and CYP153 genes were detected by real-time qPCR. The results indicated that CYP153 gene was dramatically induced by C14, C15, C16, C26 and pristane. However, alkB gene expression was only weakly detected in the presence of C14, C15 and pristane, while it was moderately induced by C16 (6.4-fold) and C26 (4.6-fold) (Fig. 7). This interesting result may be explained that the CYP153 gene likely play the predominant role in alkane degradation by Dietzia sp. CN-3, which is particularly different from previous reports (Alonso-Gutierrez et al., 2011). For example, real-time qPCR analysis showed that alkB and CYP153 expression was significantly induced by C14, C16 and pristane, and the transcript levels of the two genes were all >20-fold induced in Dietzia maris As-13-3 (Wang et al., 2014). In another report, the transcription of the alkB and CYP153 genes were induced significantly by C12–C16 n-alkanes, but there was no obvious response to C28 in the Alcanivorax hongdengensis strain A-11-3 (Wang and Shao, 2012).

From another point of view, the transcript levels of the CYP153 gene increased significantly with the increasing chain length in the n-alkanes (C14, C15, C16 and C26), reaching 86-fold induction with the C26 substrate. Notably, the transcription of the alkB gene showed a trend that was consistent with that of the CYP153 gene. Furthermore, this result was similar to Dietzia sp. E1, suggesting that the transcript levels of the alkB gene increased with the increasing length of the n-alkane chain, including C12, C16, C18 and C20 (Bihari et al., 2011). Although the alkB and CYP153 genes are frequently reported to be related to the degradation of small- and medium-chain-length alkanes (C5–C17) (Rojo, 2009), Dietzia sp. CN-3 also utilized long-chain-length alkanes (C18–C31) well.
Table 2 and the transcription of the two genes was highly induced by C26 (Fig. 7). These results suggested that the two genes, especially the CYP153 gene, may be involved in the long-chain-length alkane C26 degradation pathway in Dietzia sp. CN-3 (Wang and Shao, 2012). Further study to interpret the mechanism of hydrocarbons degradation would be the focus of our future work.

4. Conclusion

In this study, a Dietzia strain, designated as CN-3, was isolated from petroleum-contaminated sediment in Bohai Bay, China. Strain CN-3 was able to grow on C4-18 alkanes, aromatic compounds and crude oil as the sole carbon and energy sources. It efficiently degraded the saturated hydrocarbons of crude oil at a broad range of salinities and neutral to alkaline pH. Compared with the alkB gene, the CYP153 gene likely played a predominant role in medium-chain-length alkane degradation and the two genes may be related to the degradation of the long-chain-length alkane C26. Revealing the functions of this bacterium might provide important information on the indigenous bacterial strains at the contaminated sites in Bohai bay and the possible bioremediation techniques in the particular polluted areas.

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