微生物学报 *Acta Microbiologica Sinica*
55(6):755 - 763; 4 June 2015
ISSN 0001 - 6209; CN 11 - 1995/Q
http://journals.im.ac.cn/actamicrocn
doi: 10.13343/j.cnki.wsxb.20140497

Isolation and characterization of *Pseudomonas aeruginosa* strain SJTD-2 for degrading long-chain *n*-alkanes and crude oil

Jing Xu, Huan Liu, Jianhua Liu, Rubing Liang*

State Key Laboratory of Microbial Metabolism; School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China

Abstract: [**Objective**] Oil pollution poses a severe threat to ecosystems, and bioremediation is considered as a safe and efficient alternative to physicochemical. [**methods**] for eliminating this contaminant. In this study, a gram-negative bacteria strain SJTD-2 isolated from oil-contaminated soil was found capable of utilizing *n*-alkanes and crude oil as sole energy sources. The efficiency of this strain in degrading these pollutants was analyzed. [**Methods**] Strain SJTD-2 was identified on the basis of its phenotype, its physiological features, and a comparative genetic analysis using 16S rRNA sequence. Growth of strain SJTD-2 with different carbon sources (*n*-alkanes of different lengths and crude oil) was assessed, and the gas chromatography-mass spectrometry method was used to analyze the degradation efficiency of strain SJTD-2 for *n*-alkanes and petroleum by detecting the residual *n*-alkane concentrations. [**Results**] Strain SJTD-2 was identified as *Pseudomonas aeruginosa* based on the phenotype, physiological features, and 16S rRNA sequence analysis. This strain can efficiently decompose medium-chain and long-chain *n*-alkanes (C_{10}-C_{26}), and petroleum as its sole carbon sources. It preferred the long-chain *n*-alkanes (C_{18}-C_{22}), and *n*-docosane was considered as the best carbon source for its growth. In 48 h, 500 mg/L n-docosane could be degraded completely, and 2 g/L *n*-docosane was decomposed to undetectable levels within 72 h. Moreover, strain SJTD-2 could utilize about 88% of 2 g/L crude oil in 7days. Compared with other alkane-utilizing strains, strain SJTD-2 showed outstanding degradation efficiency for long-chain *n*-alkanes and high tolerance to petroleum at elevated concentrations. [**Conclusion**] The isolation and characterization of strain SJTD-2 would help researchers study the mechanisms underlying the biodegradation of *n*-alkanes, and this strain could be used as a potential strain for environmental governance and soil bioremediation.

Keywords: biodegradation, *Pseudomonas aeruginosa*, long-chain *n*-alkanes, crude oil, bioremediation
CLC number: X172　Article ID: 0001 - 6209(2015)06-0755-09

Supported by the National Science Foundation of China (31370152), by the Shanghai Pujiang Program (14PJD020) and by the Chen Xing Grant of Shanghai Jiao Tong University

* Corresponding author. E-mail: icelike@sjtu.edu.cn

Received: 18 October 2014/ Revised: 11 December 2014

Petroleum contamination from oil spills or underground storage tank leaks present a severe threat to ecosystems and result in significant environmental problems [1]. In April 2010, The "Deepwater Horizon" drilling platform located in the Gulf of Mexico exploded; the oil leak caused the world's largest accidental release of oil, and the resulting pollution belt stretched over 100 km [2]. Despite considerable efforts, a substantial portion of the oil still remains in the coastal ecosystem.

As the dissolution of hydrocarbons from crude oil in water is extremely difficult, natural remediation or chemical treatment is not sufficient to clean the oil pollution. Bioremediation, which is pollution remediation by microorganisms with oil-degrading ability, has become a very important alternative technology because of its high efficiency, low cost, and minimal secondary contamination to the environment in the elimination of oil contaminants [2]. Several researchers have tried to build efficient and stable biocatalysts to remove oil residues, and a large number of microorganisms with bioremediation potential have been reported.

Petroleum hydrocarbons include mono-aromatic hydrocarbons such as toluene, polynuclear aromatic hydrocarbons, and aliphatic hydrocarbons such as the *n*-alkanes [3]. Saturated *n*-alkanes are very important components in crude oil, accounting for approximately 20% - 50% of the total oil, and are considered as the major pollutants. As alkanes are non-polar molecules with very low chemical activity, their utilization by microorganisms faces significant challenges, owing to factors such as low water solubility, high degree of accumulation in cell membranes, and higher activation energies [4]. However, hydrocarbon-degrading microorganisms are ubiquitous in the environment, and degradation of *n*-alkanes by microorganisms is a very common phenomenon [5-9]. On the other hand, *n*-alkanes could be important carbon and energy sources for the growing microorganisms and could be transformed into pollution-free substances. Several microorganisms capable of degrading *n*-alkanes of varying lengths have been reported, including *Alcanivorax* [10], *Marinobacter* [11], *Cycloclasticus* [12], *Rhodococcus* [13], *Acinetobacter* [14], and *Pseudomonas* [15]; however, most of them can only utilize a narrow range of substrates. For example, *A. borkumensis* AP1, SK2, and SK7 can only utilize alkanes ranging from C_6 to C_{16} [16]. Many strains can utilize the short-chain or medium-chain *n*-alkanes; however, they have difficulties in breaking down the long-chain *n*-alkanes present in refractory oil residues [17].

In this study, a new *Pseudomonas aeruginosa* strain, namely, SJTD-2, was isolated from the oil-polluted soil, and its hydrocarbon utilization capability, *n*-alkane breakdown efficiency and crude oil tolerance were investigated.

1 Materials and Methods

1.1 Chemicals and media

The chemical *n*-decane (> 99% pure) was purchased from Alfa Aesar Organic Co., Inc (Tianjing, China); *n*-dodecane, *n*-tetradecane, *n*-hexadecane, and *n*-octadecane (all > 99% pure) were purchased from Sangon (Shanghai, China); and *n*-pentadecane, *n*-eicosane, *n*-docosane, *n*-tetracosane, *n*-triacontane, and *n*-hexane (of HPLC gradient grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used in this study were analytical grade reagents.

Luria-Bertani (LB) medium (tryptone 10.0 g/L, yeast extract 5.0 g/L, NaCl 10 g/L) and basal salt medium (BSM) (K_2HPO_4 3.815 g, KH_2PO_4 0.5 g, $(NH_4)_2HPO_4$ 0.825 g, KNO_3 1.2625 g, Na_2SO_4 0.2 g, $CaCl_2$ 0.02 g, $FeCl_3$ 0.002 g, and $MgCl_2$ 0.02 g/L) were used in this study. To examine the utilization of *n*-alkanes by strain SJTD-2, both liquid and solid alkanes were maintained at room temperature. The C_{10} - C_{18} alkanes were first dissolved in *n*-hexane to form 500 mg/mL alkane-hexane solutions, and the concentration of the C_{20} - C_{24} alkane-hexane solutions was adjusted to 100 mg/mL. These solutions were then added to the BSM medium to

attain various concentrations. The *n*-hexane was neither toxic to the strain, nor was it utilized by the strain.

1.2 Strain isolation and enrichment

The oil-contaminated soil from Daqing Oil Field, China was used for bacterial enrichment. No specific permissions were required, as this work did not involve any endangered or protected species. Approximately 5 g of the soil sample was inoculated into a 500 mL flask with 100 mL BSM liquid medium containing 2 g/L *n*-docosane, and the culture was shaken at 180 r/min for seven days at 30℃. A 5 mL culture was then inoculated into 100 mL of fresh BSM liquid medium with *n*-docosaneand cultured as described above. After several rounds of enrichment, the cultures were diluted and plated onto BSM agar plates pre-coated with *n*-docosane. Bacterial colonies with varying morphologies were tested for their *n*-alkane-utilizing capabilities. One strain that exhibited the fastest growth rate was purified and designated as SJTD-2.

1.3 16S rRNA gene analysis and phylogenic tree construction

The morphological, physiological, and biochemical properties of strain SJTD-2 were analyzed according to the standards listed in Bergey's Manual of Determinative Bacteriology[18]. Next, we extracted the genomic DNA of strain SJTD-2 with standard molecular biology techniques[19], and amplified the 16S rRNA gene using Bacterial 16S rDNA Kit (TaKaRa Biotechnology Co., Ltd. Dalian, China). DNA was denatured at 94℃ for 5 min, followed by denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min, and elongation at 72℃ for 1.5 min for 30 cycles, and then elongation at 72℃ for another 5 min. Subsequently, the fragments were sequenced with primers 16S-seq-F/R (16S-seq-F: 5′-GAGCGGATAACAATTTCACACAGG-3′ and 16S-seq-R: 5′-CGCCAGGGTTTTCCCAGTCACGAC-3′). The 16S rRNA gene sequence of strain SJTD-2 was deposited into GenBank (Accession No. JQ951927.1). A phylogenic tree based on the 16S rRNA sequence of strain SJTD-2 and other bacteria strains was analyzed by MEGA 5.0 using the Neighbor Joining method with 1000 replicates. The genetic distances were calculated with the Kimura two-parameter distance model.

1.4 The growth curve determination of strain SJTD-2 in different carbon sources

A single colony of strain SJTD-2 was inoculated into 100 mL LB broth in a 500 mL baffled flask, and cultured in a rotary shaker at 30℃ overnight. The cells were harvested by centrifugation for 5 min at 8000 r/min and washed thrice with sterilized water followed by re-suspension in BSM medium to $OD_{600} \approx 2.0$ to form the inoculums. Subsequently, the cell pellets were inoculated into 200 mL BSM medium supplemented with pure *n*-alkanes (C_{10}-C_{24}) of different concentrations in a 1000 mL flask. The initial cell concentration at OD_{600} was 0.1. All *n*-alkanes were prepared as *n*-hexane solutions. Cultures without *n*-alkanes and those containing *n*-alkanes without inoculum were used as controls. All the cultures were incubated at 30℃ with constant shaking at 120 r/min for seven days. The cell densities were measured by OD_{600} readings every 12 h. All experiments were repeated thrice, and the results shown were the average values of three replicates, along with the standard errors.

1.5 Analysis of SJTD-2 degradation ofpure *n*-alkanes as its sole carbon source

The degradation efficiencies of strain SJTD-2 for *n*-alkanes of different carbon lengths (C_{10} - C_{24}) were determined according to the loss of substrate, as previously described[20]. The SJTD-2 cell inoculum was prepared as described above. The cells were distributed in flasks containing 100 mL BSM medium, amended with 500 mg/L of C_{10} - C_{18} *n*-alkanes or 100 mg/L of C_{20} - C_{24} *n*-alkanes as the sole carbon source, and then were cultured for seven days at 30℃ in the shaker. The initial OD_{600} was 0.1. Flasks without cells were used as blanks to assess the abiotic loss. The cultures were taken out at different time points to estimate the cell concentrations and the alkane residues. For the residual analysis of alkanes, 1 mL of cell culture was collected and divided into two

1.5 mL Eppendorf tubes, and subsequently extracted with 250 μL n-hexane. The mixtures were shaken vigorously for 2 min, and centrifuged at 12000 r/min for 2 min. Then the organic layer was collected and the aqueous layer was extracted three more times, with 250 μL n-hexane each time. The organic extracts were pooled together to a final volume of 1 mL and dried with anhydrous sodium sulfate. For each sample, *n*-pentadecane (100 mg/L) was added before the extraction and used as an internal standard. All extractions were performed in triplicate, and the results were expressed as average values with standard errors.

The degradation efficiencies of strain SJTD-2 for *n*-docosane of various concentrations (250 mg/L, 500 mg/L, 1000 mg/L, and 2000 mg/L) were also analyzed as above. After strain SJTD-2 was cultured for 1 – 7 days, the residual *n*-hexadecane was extracted at different time points with three replicates each.

1.6 Analysis of *n*-alkane concentration

The concentrations of *n*-alkanes were determined by the gas chromatography-mass spectrometry (GC-MS) technique, using a GC/MS system (7890A GC/5975C MS, Agilent Technologies, USA) equipped with a fused DB-5 MS capillary column (0.25 mm × 30 m × 0.25 μm) [20]. The GC program was listed as below. Helium was used as the carrier gas with a constant flow rate of 1.0 mL/min. The split ratio was 10:1, and the injector and connector temperatures were 270℃ and 280℃, respectively. The temperatures of the ion source and the quadrupole were 230℃ and 150℃, respectively. The column oven temperature was maintained at 150℃ for 2 min, then increased to 200℃ at a rate of 5℃/min, followed by an increase to 290℃ at a rate of 30℃/min, and at an isotherm of 290℃. The ionization mode was set as EI +, 70 eV, and the voltage of the detector was 1388 V. The GC-MS spectra were analyzed with ChemStation software, and the relative abundance of different hydrocarbon residues was calculated by the ratio of the peak area of each hydrocarbon to the peak area of *n*-pentadecane in the GC chromatograph. The residue ratio of *n*-alkanes was calculated with the equation $R = [S] / [I]$, where R, $[S]$, and $[I]$ represent the alkane residue ratio, the residual *n*-alkane concentrations in the samples, and the concentration before inoculation, respectively. The results were expressed as mean values with standard deviations. The cell-free controls were incubated and analyzed in the same manner.

2 Results and Discussion

2.1 Isolation and identification of oildegradation strain SJTD-2

Strain SJTD-2, a rod-shaped gram-negative bacterium, was isolated by enriching the oil-contaminated soil. Its growth pH ranged from 3.5 to 9.5, and optimal growth occurred at pH 7.0 to 8.0. After growing on LB agar at 30℃ for 24 h, the cells formed yellow to green, round, moist, and glossy colonies, approximately 1.0 mm in diameter. Strain SJTD-2 was able to utilizen-alkanes from *n*-dodecane (C_{10}) to *n*-triacontane (C_{26}) as its sole carbon sources. Although SJTD-2 failed to grow in the presence of shorter length *n*-alkanes, its growth was not inhibited by short-chain *n*-alkanes such as *n*-hexane. The 16S rRNA gene sequence of strain SJTD-2 (GenBank Accession No. JQ951927.1) was 99% identical to that of *P. aeruginosa* PAO1 (GenBankAccession No. DQ777865.1), and the corresponding phylogenetic analysis supported a close relationship between SJTD-2 and members of the genus *Pseudomonas* sp. (Figure 1). Therefore, SJTD-2 was classified as a *P. aeruginosa* strain.

2.2 Growth curve analysis of strain SJTD-2 with n-alkanes as its sole carbon source

The utilization capability and efficiency of strain SJTD-2 with regard to *n*-alkanes was studied by monitoring the cell growth for seven days in BSM medium with 500 mg/L of differentn-alkanes (C_{10} – C_{24}). The growth plot showed the cell densities at different time points (Figure 2).

From the growth curve, we found that strain SJTD-2 utilized the long-chain *n*-alkanes more efficiently than the medium-chain and short-chain *n*-

85 *Pseudomonas chlororaphis* subsp.*chlororaphis* DSM 500883T (NR 044974.1)
82 *Pseudomonas chlororaphis* subsp.*aureofaciens* DSM 6698 (NR 042939.1)
69 *Pseudomonas fluorescens* IAM 12022 (NR 043420.1)
Pseudomonas sp. PM-2001 (AF 321239.1)
85 *Pseudomonas libanensis* CIP 105460 (NR 024901.1)
94 99 *Pseudomonas fulgida* MSB2111 (AY 275485.1)
72 *Pseudomonas mandelii* CIP 105273 (NR 024902.1)
Pseudomonas syringae MSB2018 (AY 275478.1)
32 99 *Pseudomonas* sp. EC-V30-2 (AB 379689.1)
64 *Pseudomonas syringae* LMtotH4 (DQ 870717.1)
Pseudomonas rhizosphaerae IH5 (NR 029063.1)
42 *Pseudomonas argentinensis* CH01 (NR 043115.1)
71 *Pseudomonas plecoglossicida* FPC951 (NB 024662.1)
27 *Pseudomonas putida* GPoI (AJ249825.1)
67 *Pseudomonas putida* CB2-1 (GQ240852.1)
34 *Pseudomonas putida* DSM 3602 (AF509331.1)
78 *Pseudomonas putida* YJF3-34 (FJ405889.1)
79 *Pseudomonas xanthomarina* KMM 1447 (NR 041044.1)
Pseudomonas alcaliphila AL15-21 (NR 024734.1)
25 *Pseudomonas pseudoalcaligenes* Stanier 63 (NR 037000.1)
32 *Pseudomonas mendocina* NCIB 10541 (NR 043421.1)
35 *Pseudomonas* sp. WBUNB003 (JX290077.1)
Pseudomonas sp. SJTD-2 (JQ951927.1)
98 *Pseudomonas aeruginosa* DSM 50071 (NR 026078.1)
99 *Pseudomonas aeruginosa* DQ8 (GU269267.1)
89 *Pseudomonas aeruginosa* PAO1 (DQ777865.1)
40 *Pseudomonas aeruginosa* PJHU15 (JN099685.1)
0.005
substiutcs/sites

Figure 1. Phylogenetic tree based on 16S rDNA gene sequences indicating the SJTD-2 position. The Kimura two-parameter distance model was used and bootstrap analysis was performed with 1000 replicates using MEGA 5.0 software. Numbers in parentheses represent the sequences' accession number in GenBank. The number at each point is the percentage supported by bootstrap. Bar, 0.5% sequence divergence.

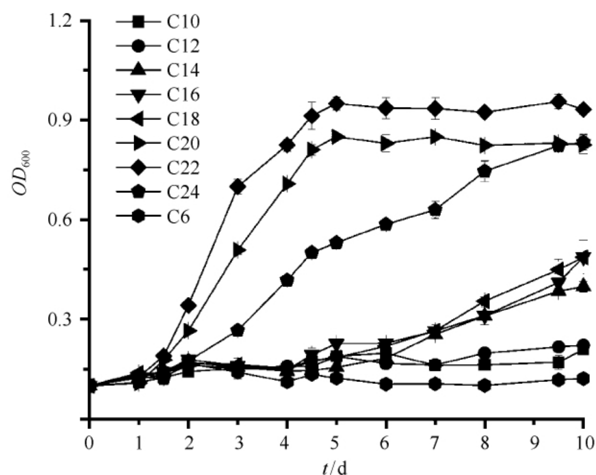

Figure 2. Growth curves of strain SJTD-2 in 500 mg/L n-alkanes. Strain SJTD-2 was cultured in BSM supplemented with 500 mg/L n-alkanes of different length for seven days at 30℃. Data are expressed as means and standard deviations.

alkanes. It preferred to use n-eicosane, n-docosane and n-tetracosane; n-docosane resulted in the highest cell density, followed by n-eicosane, n-tetracosane, and n-octadecane. Thus, n-docosane appeared to be the best available carbon source for strain SJTD-2 in this set of experiments. The cell utilization efficiency was low in case of n-alkanes with less than 14 carbons (Figure 2). With the long-chain n-alkanes, SJTD-2 cells started multiplying approximately 24 h after incubation, and most cultures reached the exponential growth phase between days 2 and 5. The maximum OD_{600} reached 1.0, 0.8, and 0.78, respectively, with n-docosane, n-eicosane, and n-tetracosane, although n-tetracosane required more time to develop (Figure 2). Although strain SJTD-2 grew well with long-chain n-alkanes, it still required a longer duration time to

utilize the very long-chain *n*-alkanes. An advantage of strain SJTD-2 in the degradation of long-chain *n*-alkanes is that is can utilize crude oil as a carbon source, making it a potential bioremediation strain for environmental oil pollution.

2.3 Degradation efficiency of strain SJTD-2 in alkanes and crude oil

To determine the highest concentration of *n*-alkanes that strain SJTD-2 could tolerate and utilize for growth, we analyzed its degradation efficiency forn-docosane (concentrations ranging from 250 mg/L to 2.0 g/L) by detecting the concentration of residual *n*-docosane through GC-MS analysis, asn-docosane was considered its best carbon source. As shown in Fig. 3, *n*-hexadecane was completely degraded in three days. Additionally, 99% of the 250 mg/L and 500 mg/L of *n*-docosane were degradedwithin 48 h. Approximately one more day was required for the complete degradation of 1.0 g/L and 2.0 g/L *n*-docosane, and about 70% and 35% of *n*-docosane at these two concentrations could be utilized in the first 48 h. Therefore, we concluded that strain SJTD-2 could tolerate and completely biotransform 2.0 g/L *n*-docosane in just three days and produce a relatively large amount of biomass, implying its high degradation efficiency of long-chain alkanes.

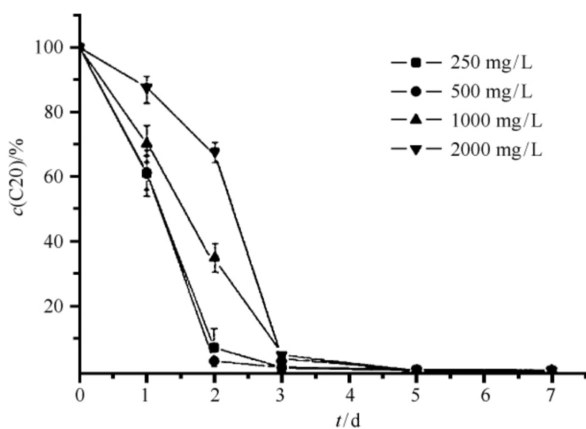

Figure 3. Degradation efficiency of strain SJTD-2 in *n*-docosane of different concentrations. The strain was cultured in BSM supplemented with *n*-docosaneof different concentrations at 30℃ for seven days. Standard errors were calculated from three independent determinations.

Furthermore, we analyzed the utilization efficiency of strain SJTD-2 with 2.0 g/L crude oil as carbon source. Although the degradation in the first 24 h was slow, about 88% of the crude oil was transformed completely by this strain in seven days (Figure 4). These results proved that strain SJTD-2 could tolerate long-chain *n*-alkanes and crude oil of high concentrations and that it biotransformed them efficiently.

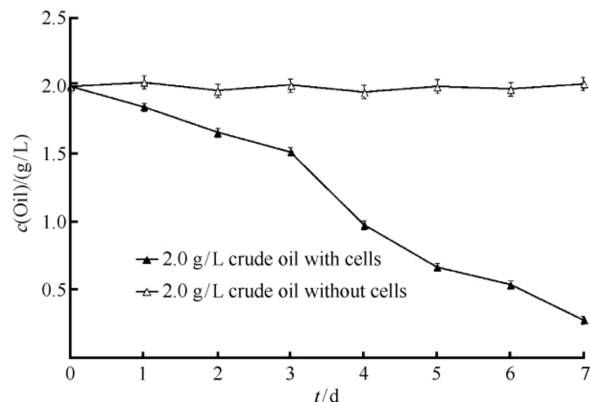

Figure 4. Quantitative estimation of the efficiency of strain SJTD-2 to utilize 2 g/L crude oil. Strain SJTD-2 was cultured in BSM supplemented with 2g/L crude oil for seven days and the residual oil concentration was detected. Crude oilwithout cells was used the abiotic controls. Standard errors were calculated from three independent determinations.

In the past 30 years, at least sixty genera of aerobic bacteria, such as *Pseudomonas* [15], *Acinetobacter* [14], *Rhodococcus* [13] and *Dietzia* [21], and five genera of anaerobic bacteria have been reported to possess the ability to degrade aliphatic hydrocarbons. Among them, *Pseudomonas* was found in soil as well as in aqueous environments [22]. Several *Pseudomonas* strains are known to use aliphatic hydrocarbons as their sole carbon sources [23-25]. *P. aeruginosa* RR1 and *P. fluorescens* CHA0 degrade *n*-alkanes ranging from C_{12} to C_{34} [26-27], and *P. aeruginosa* DQ8 can grow in the presence of *n*-tetrodecane, *n*-docosane, *n*-triacontane, and *n*-tetrocontane [28]. Although several strains have been reported to utilize hydrocarbons, a majority of them can use only a narrow range of substrates. Indeed, very few strains, including *Acinetobacterbaylyi* ADP1 and *Thermus*sp. C2, can degrade a wide range

of hydrocarbons. The genetic characteristics of these bacteria, however, remain elusive, and not much is known about the mechanisms by which these microorganisms break down long chain alkanes present in refractory oil residues [17, 29].

In this study, we determined a novel *n*-alkanes-degrading bacteria strain that could efficiently utilize the medium-chain *n*-alkanes, the long-chain *n*-alkanes, and crude oil as its sole carbon source. We observed that this strain preferred to utilize the long-chain *n*-alkanes such as *n*-eicosane, *n*-docosane and *n*-tetracosane, and *n*-docosane as its best carbon sources for growth. In 48 h, 500 mg/L *n*-docosane could be degraded completely, while 2 g/L *n*-docosane was decomposed to undetectable levels within 72 h. Moreover, strain SJTD-2 could biotransform about 88% of the 2 g/L crude oil in seven days. Although a relatively narrower substrate range of C_{10} – C_{26} supported a sustainable growth of strain SJTD-2, it showed outstanding degradation efficiency and utilization speed for the long-chain *n*-alkanes. Thus, *n*-docosane followed by *n*-eicosane, *n*-tetracosane, and *n*-octadecane were assimilated as preferential carbon sources for this strain. Compared with other alkane-utilizing strains, strain SJTD-2can efficiently degrade many more *n*-alkanes withmuch longer carbon chains in a shorter time (Table 1). Only this strain was found to be capable of completely degrading 500 mg/L of n-docosane in 48 h. The best carbon sources of most of the alkane-consuming strains are the medium-chain *n*-alkanes ($\leqslant C_{18}$), and compared with strain SJTD-2, these strains mineralize the equivalent amounts of *n*-alkanes at a much slower rate and require a much longer degradation time (Table 1). In the similar culture conditions, *Alcanivorax* sp. 2B5 consumed only 16.07% of the 500 mg/L of *n*-octadecane in 48 h. *P. aeruginosa* DQ8 utilized 100 mg/L of *n*-octadecane in seven days with the addition of other carbon sources. Although another *P. aeruginosa* strain SJTD-1 was reported as capable of utilizing 500 mg/L of *n*-octadecane in 36 h, the efficient utilization of *n*-alkanes ($\geqslant C_{20}$) by this strain was difficult, and only about 48% of the 500 mg/L of *n*-docosane was decomposed in 96 h (Liu *et al.*, 2014). Compared with strain *Dietzia* DQ12-45-1b, strain SJTD-2 exhibited rapid adaptation to the very high concentration of *n*-octadecane ($\geqslant$ 2 g/L), showing much faster degradation and greater utilization efficiency (Table 1).

Table 1. Comparison of *n*-alkanes degradation efficiencies of different bacteria strains

Strains	*n*-alkanes	Rate	Sources
P. aeruginosa SJTD-2	C_{22}, 500 mg/L	>99% in 48 h	Present study
	C_{22}, 2.0 g/L	>96% in 72 h	
P. aeruginosa SJTD-1	C_{22}, 500 mg/L	48% in 96h	[20]
P. aeruginosa DQ8	C_{22}, 100 mg/L	>99% in 7 d*	[28]
Alcanivorax sp. 2B5	C_{18}, 500 mg/L	16.07% in 48h	[30]
Acinetobacter	C_{16}, 400mg/L	>99% in 60h	[31]
*Dietzia*DQ12-45-1b	C_{20}, 2.5g/L	8.9% in 21d	[21]

* added with 0.005% yeast extract in medium.

Acknowledgements: Thanks for Jian He of Nanjing Agricultural University for his support in isolation of this strain, and thanks for Wenjuan Yu of Instrumental Analysis Center of SJTU for her support in the GC-MS detection.

References

[1] Allen JP, Atekwana EA, Atekwana EA, Duris JW, Werkema DD, Rossbach S. The microbial community structure in petroleum-contaminated sediments corresponds to geophysical signatures. *Applied and Environmental Microbiology*, 2007, 73(9): 2860-2870.

[2] Kostka JE, Prakash O, Overholt WA, Green SJ, Freyer G, Canion A, Delgardio J, Norton N, Hazen TC, Huettel M. Hydrocarbon-degrading bacteria and the bacterial community response in gulf of Mexico beach sands impacted by the deepwater horizon oil spill. *Applied and Environmental Microbiology*, 2011, 77(22): 7962-7974.

[3] Wasmund K, Burns KA, Kurtböke DI, Bourne DG. Novel alkane hydroxylase gene (alkB) diversity in sediments associated with hydrocarbon seeps in the Timor sea, Australia. *Applied and Environmental Microbiol*ogy, 2009, 75(23): 7391-7398.

[4] Kaplan CW, Kitts CL. Bacterial succession in a petroleum land treatment unit. *Applied and Environmental Microbiol*ogy, 2004, 70(3): 1777-1786.

[5] Labinger JA, Bercaw JE. Understanding and exploiting C-H bond activation. *Nature*, 2002, 417: 507-514.

[6] Head IM, Jonesm DM, Röling WF. Marine microorganisms make a meal of oil. *Nature Review Microbiology*, 2006, 4: 173-182.

[7] Alonso-Gutiérrez J, Figueras A, Albaigés J, Jiménez N, Viñas M, Solanas AM, Novoa B. Bacterial communities from shoreline environments (costa da morte, northwestern Spain) affected by the prestige oil spill. *Applied and Environmental Microbiol*ogy, 2009, 75(11): 3407-3418.

[8] Rojo F. Degradation of alkanes by bacteria. *Environmental Microbiology*, 2009, 11: 2477-2490.

[9] Hazen TC, Dubinsky EA, DeSantis TZ, Andersen GL, Piceno YM, Singh N, Jansson JK, Probst A, Borglin SE, Fortney JL, Stringfellow WT, Bill M, Conrad ME, Tom LM, Chavarria KL, Alusi TR, Lamendella R, Joyner DC, Spier C, Baelum J, Auer M, Zemla ML, Chakraborty R, Sonnenthal EL, D'haeseleer P, Holman HY, Osman S, Lu Z, Van Nostrand JD, Deng Y, Zhou J, Mason OU. Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science*, 2010, 330: 204-208.

[10] Liu C, Shao Z. *Alcanivoraxdieselolei* sp. nov., a novel alkane-degrading bacterium isolated from sea water and deep-sea sediment. *International Journal of Systematic and Evolutionary Microbiology*, 2005, 55(Pt 3): 1181-1186.

[11] Gauthier MJ, Lafay B, Christen R, Fernandez L, Acquaviva M, Bonin P, Bertrand JC. Marinobacterhydrocarbonoclasticus gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. *International Journal of Systematic and Evolutionary Microbiology*, 1992, 42(4): 568-576.

[12] Maruyama A, Ishiwata H, Kitamura K, Sunamura M, Fujita T, Matsuo M, Fujita T, Higashihara T. Dynamics of microbial populations and strong selection for *Cycloclasticuspugetii* following the Nakhodka oil spill. *Microbial Ecology*, 2003, 46: 442-453.

[13] Kim D, Kim YS, Kim SK, Kim SW, Zylstra GJ, Kim YM, Kim E. Monocyclic aromatic hydrocarbon degradation by *Rhodococcus* sp. strain DK17. *Applied and Environmental Microbiol*ogy, 2002, 68(7): 3270-3278.

[14] Tani A, Ishige T, Sakai Y, Kato N. Two acyl-CoA dehydrogenases of *Acinetobacter* sp. strain M-1 that uses very long-chain n-alkanes. *Journal of Bioscience and Bioengineering*, 2002, 94(4): 326-329.

[15] Whyte LG, Bourbonniére L, Greer CW. Biodegradation of petroleum hydrocarbons by psychrotrophic*Pseudomonas* strains poccessingboth alkanes (*alk*) and naphthalene (*nah*) catabolic pathway. *Applied and Environmental Microbiol*ogy, 1997, 63(9): 3719-3723.

[16] van Beilen JB, Marin MM, Smits TH, Röthlisberger M, Franchini AG, Witholt B, Rojo F. Characterization of two alkane hydroxylase genes from the marine hydrocarbonoclastic bacterium *Alcanivorax borkumensis*. *Environmental Microbiology*, 2004, 6: 264-273.

[17] Hao R, Lu A, Wang G. Crude-oil-degrading thermophilic bacterium isolated from an oil field. *Canadian Journal of Microbiology*, 2004, 50: 175-182.

[18] Holt J, Krieg N, Sneath P, Staley J, Williams S. International edition: Bergey's Manual of Determinative Bacteriology. Baltimore: Williams & Wilkins, 1994.

[19] Joseph S, David W. Molecular cloning: a laboratory manual. 8th eds. New York: Cold Spring Harbor Laboratory Press, 2001.

[20] Liu H, Xu J, Liang R, Liu J. Characterization of the medium-and long-chain n-alkanes degrading *Pseudomonas aeruginosa* strain SJTD-1 and its alkane hydroxylase genes. *PLoS One*, 2014, 9(8): e105506.

[21] Wang XB, Chi CQ, Nie Y, Tang YQ, Tan Y, Wu G, Wu XL. Degradation of petroleum hydrocarbons (C6-C40) and crude oil by a novel *Dietzia* strain. *Bioresource Technology*, 2011, 102: 7755-7761.

[22] Spiers AJ, Buckling A, RaineyPB. The causes of *Pseudomonas* diversity. *Microbiology*, 2000, 146: 2345-2350.

[23] Alonso A, Rojo F, Martínez JL. Environmental and clinical isolates of *Pseudomonas aeruginosa* show pathogenic and biodegradative properties irrespective of their origin. *Environmental Microbiology*, 1999, 1: 421-430.

[24] van BeilenJB, Veenhoff L, Witholt B. New frontiers in screening formicrobialbiocatalysts. //Kieslich K, van der Beek CP, de Bont JAM, van den TweelWJJ. Alkane Hydroxylase Systems in *Pseudomonas aeruginosa* Strains

able to Grow on n-octane. AmsterdamNetherlands: Elsevier Science BV, 1998: 211-215.

[25] van Beilen JB, Wubbolts MG, Witholt B. Genetics of alkane oxidation by *Pseudomonas oleovorans*. *Biodegradation*, 1994, 5: 161-174.

[26] Yuste L, Corbella MaE, Turiégano MaJ, Karlson U, Puyet A, Rojo F. Characterization of bacterial strains able to grow on high molecular mass residues from crude oil processing. *FEMS Microbiology Ecology*. 2000, 32: 69-75.

[27] Smits TH, Balada SB, Witholt B, van Beilen JB. Functional analysis of alkane hydroxylases from gram-negative and gram-positive bacteria. *Journal of Bacteriology*, 2002, 184: 1733-1742.

[28] Zhang Z, Hou Z, Yang C, Ma C, Tao F, Xu P. Degradation of n-alkanes and polycyclic aromatic hydrocarbons in petroleum by a newly isolated *Pseudomonas aeruginosa* DQ8. *Bioresource Technology*, 2011, 102: 4111-4116.

[29] Santos PM, Benndorf D, Sa-Correia I. Insights into *Pseudomonas putida* KT2440 response to phenol-induced stress by quantitative proteomics. *Proteomics*, 2004, 4: 2640-2652.

[30] Liu YC, Li LZ, Wu Y, TianW, Zhang LP, Xu L, Shen QR, Shen B. Isolation of an alkane-degrading *Alcanivorax* sp. strain 2B5 and cloning of the *alkB* gene. *Bioresource Technology*, 2010, 101: 310-316.

[31] Sun JQ, XuL, Tang YQ, Chen FM, Wu XL. Simultaneous degradation of phenol and *n*-hexadecane by *Acinetobacter* strains. *Bioresource Technology*, 2012, 123: 664-668.

铜绿假单胞菌 SJTD-2 降解长链烷烃与原油的特性

徐静,刘环,刘建华,梁如冰*

微生物代谢国家重点实验室,上海交通大学生命科学技术学院,上海 200240

摘要:【目的】石油污染严重威胁生态系统和生物圈,微生物修复被认为是一种安全有效可代替物化方法来治理石油污染的办法。本文对我们从石油污染土壤中分离获得的一株可分解正烷烃和原油的革兰氏阴性菌 SJTD-2 的理化性质和降解效能进行了研究。【方法】利用菌株表型和生理性质、16S rRNA 序列比较分析与进化树绘制,确定新分离菌株 SJTD-2 的种属;测定菌株 SJTD-2 的生长曲线,确定其利用不同长度烷烃和原油为单一碳源的效能;利用 GC-MS 检测烷烃类物质的残留量,确定菌株 SJTD-2 降解烷烃和原油 SJTD-2 的降解效率和降解周期。【结果】菌株表型与 16S rRNA 序列比较及进化树比对分析结果显示,菌株 SJTD-2 与假单胞菌属的亲缘关系十分接近,为铜绿假单胞菌。菌株 SJTD-2 可有效分解 C_{10} 到 C_{26} 的中链和长链烷烃及原油,利用它们作为其单一碳源生长;该菌株对长链烷烃($C_{18}-C_{22}$)的利用效果较中链烷烃好,其中正二十二烷被认为是其最佳碳源。48 h 内,该菌株可完全降解 500 mg/L 正二十二烷;72h 后,2 g/L 的正二十二烷可几乎被菌株全部分解利用。此外,菌株 SJTD-2 在 7 d 内可将 2 g /L 的原油分解 88% 以上。【结论】与现有其它烷烃降解菌相比,铜绿假单胞菌 SJTD-2 具有突出的长链烷烃与原油降解效能及耐受能力,该菌株的发现与研究将有助于烷烃降解机制的研究和环境修复的进程。

关键词:生物降解,铜绿假单胞菌,长链烷烃,原油,生物修复

中图分类号:X172 **文章编号**:0001-6209(2015)06-0763-09

(本文责编:王晋芳)

基金项目:国家自然科学基金项目(31370152);上海浦江人才计划(14PJD020);上海交通大学晨星青年学者研究计划

* 通信作者。E-mail: icelike@sjtu.edu.cn

作者简介:徐静(1989-),女,山东人,硕士研究生,研究方向为微生物分子生物学。E-mail: xjjkary1989@126.com

收稿日期:2014-10-18;修回日期:2014-12-11