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RESEARCH

In situ bioremediation of petroleum hydrocarbon–contaminated soil: isolation and application of a *Rhodococcus* strain

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Abstract

Due to low consumption and high efficiency, in situ microbial remediation of petroleum hydrocarbons (PHs)-contaminated sites in in-service petrochemical enterprises has attracted more and more attention. In this study, a degrading strain was isolated from oil depot–contaminated soil with soil extract (PHs) as the sole carbon source, identified and named *Rhodococcus* sp. OBD-3. Strain OBD-3 exhibited wide adaptability and degradability over a wide range of temperatures (15–37 °C), pH (6.0–9.0), and salinities (1–7% NaCl) to degrade 60.6–86.6% of PHs. Under extreme conditions (15 °C and 3–7% salinity), PHs were degraded by 60.6 ± 8.2% and more than 82.1% respectively. In OBD-3, the alkane monooxygenase genes *alkB1* and *alkB2* (GenBank accession numbers: MZ688386 and MZ688387) were found, which belonged to *Rhodococcus* by sequence alignment. Moreover, strain OBD-3 was used in lab scale remediation in which the contaminated soil with OBD-3 was isolated as the remediation object. The PHs were removed at 2,809 ± 597 mg/kg within 2 months, and the relative abundances of *Sphingobium* and *Pseudomonas* in soil increased more than fivefold. This study not only established a system for the isolation and identification of indigenous degrading strains that could efficiently degrade pollutants in the isolated environment but also enabled the isolated degrading strains to have potential application prospects in the in situ bioremediation of PHs-contaminated soils.

Keywords Petroleum hydrocarbons · In situ bioremediation · In-service enterprises · *Rhodococcus* · Degradation characteristics

Introduction

Global oil consumption is increasing every year (Bharti et al. 2021), and the USA and China are among the top countries worldwide in terms of oil refining and oil consumption (Al-Fattah and Aramco 2021). In 2020, the USA had 135 operable refineries and approximately 115,000 gas stations (EIA 2021; Patel 2021). China also had 28 10-million-ton refining and chemical enterprises and 119,000 gas stations (Liu 2022). However, oil leaks inevitably occur during processing, storage, and use, causing serious pollution to the local or surrounding soil of in-service enterprises such as refining and chemical enterprises, oil depots, and gas stations (Liu et al. 2015; Wu et al. 2022). Petroleum hydrocarbons (PHs) are typical pollutants in oil-contaminated soils. In the Dagang Oilfield of China, the concentrations of total petroleum hydrocarbons (TPHs) in soils collected from oil refineries and transportation zones ranged from $(2.0 \pm 0.5) \times 10^4$ to $(2.3 \pm 0.2) \times 10^4$ mg kg^{-1} dm (Liu et al. 2015). At a gas station in Nanjing, the C10–C40 hydrocarbons concentrations were high in most samples, especially at shallow depths, reaching 3680 mg/kg (Wu et al. 2022). In addition, when a sufficient amount of PHs was released on the (underground) surface, they migrated vertically downward until reaching the groundwater and then spread laterally (Banerji et al. 1995), causing serious pollution to

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groundwater and even surrounding rivers or waters. At the abovementioned Nanjing gas station, the maximum concentration of TPHs in groundwater was 13.1 mg/L (Wu et al. 2022). In the shallow groundwater around the gas station in Chongqing, the detection rate of PHs was 96.3% (Zhao et al. 2016). PHs pose high potential risks and harmful effects to humans and other organisms surrounding contaminated aquatic and terrestrial ecosystems (Haider et al. 2021). Therefore, the PHs pollution of in-service petrochemical enterprises urgently needs to be remediated.

The contaminated sites of in-service enterprises could be remediated by in situ remediation, mainly due to it was deeply repaired without excavation (Kuppusamy et al. 2016), to avoid affecting normal production. However, the safety requirements are high in the remediation process, and pollution easily rebounds (O'Connor et al. 2018), so it is necessary to choose a safe, efficient, and continuous in situ remediation method. Microbial remediation is a better choice due to its flexibility, efficiency, effectiveness, economy, and eco-friendliness (Azubuike et al. 2020). Bioaugmentation is a type of microbial remediation that degrades pollutants by added indigenous or allochthonous degrading microbial agents (Yu et al. 2014). The microbial agents used in remediation need to have efficient degradation activity, good environmental adaptability, and no biological risk. Therefore, the isolation of high-efficiency degrading microorganisms from contaminated soil has been the focus of research (Song et al. 2021).

At present, there are many strains that can degrade PHs, including *Pseudomonas* (Xie et al. 2011), *Rhodococcus* (Huang et al. 2008; Takei et al. 2008; Li et al. 2013; Liu et al. 2016; Hu et al. 2020), *Bacillus* (Wang et al. 2020), *Acinetobacter* (Lal and Khanna 1996), *Alcanivorax* (Hara et al. 2004), and *Sphingomonas* (Li et al. 2013; Wang et al. 2020). However, some pathogenic or opportunistic pathogens among these degrading strains were not safe for soil remediation. In addition, non-indigenous degrading strains were used to remediate soil, which might be difficult to adapt to the environment due to temperature, salinity, and other factors, resulting in no survival. Therefore, it was particularly important to isolate indigenous strains that were highly adaptable, and could be efficiently degraded and engineered.

In this study, the indigenous PHs degrading strain OBD-3 was isolated from the oil depot contaminated soil with soil extract (PHs) as the sole carbon source. OBD-3 was identified by bacterial morphology and 16S rRNA gene sequence analysis. Its degradation characteristics were investigated by degrading PHs at different medium pH, culture temperatures, and salinities. The PHs degradation genes of OBD-3 were detected by PCR amplification, and their amino acid sequences were analyzed. Thus, an isolation and identification system of indigenous degrading strains that could efficiently degrade pollutants in the isolated environment was formed. In addition, OBD-3 was utilized to remediate the contaminated soil from which it was isolated, and the effects of the remediation on the microbial community structure in the in situ soil were investigated. These findings provide strain resources and theoretical support for the in-situ bioremediation of PHs-contaminated soil.

Materials and methods

Chemicals and culture media

The PHs used in all experiments were extracted with dichloromethane, concentrated and finally dissolved in *n*-hexane to form a liquid with a density of 0.8 g/cm^3. All other reagents and solvents used were of analytical grade and the highest purity available. 1% salinity mineral salt medium (MSM) was used to isolate strains and degrade PHs, which contained the following: 0.2 g/L NH_4Cl, 7.95 g/L NaCl, 0.77 g/L $MgCl_2{\cdot}6H_2O$, 1.05 g/L $MgSO_4{\cdot}7H_2O$, 0.076 g/L $CaCl_2$, 0.22 g/L KCl, 0.01 g/L $NaHCO_3$, 0.026 g/L NaBr, 0.25 g/L K_2HPO_4, and trace element solution (1 mL/L) (Feng et al. 2012). Other salinity MSM were prepared in proportion. Luria–Bertani (LB) medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) was used for the isolation and cultivation of strains. Solid agar plates were prepared with the addition of 1.5% (w/v) agar to the LB liquid medium.

Isolation and identification of degrading strains

The soil samples for isolation of potential PHs degrading strains were collected from the contaminated soil of an oil depot in Shanghai, and were transported aseptically at 4 °C to the laboratory. A soil sample (5 g) was used to inoculate a 250-mL flask with 100 mL of MSM containing 800 mg/L PHs as the sole carbon source (Sood and Lal 2008). The flask was shaken at 28 °C, 180 r/min, in the dark and transferred every 4 days, and the inoculation volume was 10% (v/v). After multiple transfers, the remaining PHs content was determined, and the microbial community with PHs degradation ability was selected to isolate single strain. The microbial community was diluted and spread on LB solid medium and incubated at 28 °C for 2 days. Single bacterial colonies were selected to be streaked and separated on the LB agar plates, and finally, the PHs degrading single strain was obtained.

The genomic DNA of strain OBD-3 was extracted by using Fast DNA™ Spin DNA extraction kit (MP Biomedical, USA). The 16S rRNA gene was amplified from genomic DNA using the universal primers 27F and 1492R (STable 1). The *gyrB* gene was amplified from genomic DNA using *gyrB*-F and *gyrB*-R (STable 1) (Táncsics et al. 2014). The amplifications were performed using Taq DNA polymerase

under standard reaction conditions, and sequenced by Sangon Biotech Co., Ltd. Sequences were aligned and analyzed by NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast) server. The phylogenetic analysis was performed using the neighbor-joining method by MEGA X (version 10.2.6).

Degradation of PHs by degrading strain

To study the degradation characteristics of strain OBD-3, the effects of pH, temperature, and salinity on degradation were investigated. Different pH values (pH 6.0, pH 7.0, pH 8.0, pH 9.0) were tested. Strain OBD-3 was cultured in LB medium containing 1000 mg/L PHs, and shaken at 28 °C for 1 day. The cells were centrifuged at 10,000 × g for 5 min, washed with PBS twice, and transferred to 50 mL of MSM containing 1000 mg/L PHs to make the initial OD_{600} = 0.2. The degradation system was shaken at 180 r/min for 7 days in the dark. Similarly, the effects of culture temperature (15 °C, 28 °C, 37 °C) and salinity (1%, 3%, 5%, 7%) in MSM were also tested for strain OBD-3. Three parallel experiments were performed in each group, and blank control (degradation system without strain OBD-3) was performed. The residual PHs in MSM were extracted with an equal volume of *n*-hexane, and the extract was filtered through 0.22 μm millipore filter to prepare the samples for subsequent analysis by gas chromatography (GC).

The GC analysis was carried out on GC-2014 (Shimadzu company, Japan), equipped with flame ionization detector (FID), and the injection chromatographic column was HP-5 capillary column (30 m × 0.25 mm × 0.25 μm. Agilent Technology Co., Ltd.). The conditions were based on the China HJ 1021–2019 methods, as follows: inlet temperature 300 °C and detector temperature 325 °C. The program was as follows: initial temperature 50 °C for 2 min, heating at 40 °C/min to 230 °C; then, the temperature was increased at the rate of 20 °C/min up to 320 °C, with a hold time of 20 min. The column flow rate was 1.5 mL/min. The biodegradation efficiency (%) was calculated as follows: biodegradation efficiency (%) = $(C_0\text{-}C_t\text{-}C_L)/(C_0\text{-}C_L) \times 100\%$, where C_0 is the initial concentration of PHs in the culture medium, C_t is the concentration of PHs in the culture medium after degradation for a certain time, and C_L is the concentration of volatilization loss.

The detection of degradation genes

The degradation genes were amplified from genomic DNA using the primers of alkane monooxygenase genes *alkB1* and *alkB2* (Yang et al. 2015), as shown in STable 1. The amplifications were performed using Taq DNA polymerase. The annealing temperature was 62 °C, and the extension time was 1 min. The PCR products were detected by DNA gel electrophoresis and sequenced by Sangon Biotech Co., Ltd. The analysis of sequences was described in "Isolation and identification of degrading strains." Motif searches were performed using the Vector NTI AlignX software (version 11.0).

Remediation of contaminated soil

The tested soil was the contaminated soil used to isolate the degrading strain and was collected after grinding and screening (30 mesh). Strain OBD-3 was cultured as described above. The cells were centrifuged and washed to form resting cells (OD_{600} = 10.0) in 50 mM phosphate buffer (pH 7.4). The resting cells were evenly sprayed into 500 g of polluted soil according to the water soil ratio of 1:10. The experimental soil was placed in a constant-temperature incubator (25 °C) for cultivation, and a moisture content of 20–25% was maintained. The samples were taken every 30 days. The contaminated soil with deionized water was used as the control group. Three parallel experiments were performed in each group.

For the extraction of PHs in soil, 5 g of dry weight soil, dichloromethane (v:w = 4:1) as extractant, ultrasonic extraction for 10 min, centrifugation at 5000 × g for 10 min, was repeated 4 times (Guo et al. 2017). The extract was collected and concentrate it to about 1 mL, added 10 mL of *n*-hexane to concentrate to 1 mL, repeat for 2 times, and finally diluted to 1 mL with *n*-hexane. The prepared samples were diluted 100 times with *n*-hexane and passed through 0.22 μm millipore filter, and the above method was used to detect the content of PHs by GC.

Microbial community structure analysis

The initial contaminated soil (CK 0 M) and remediated soil after 2 months (E 2 M) were collected for MiSeq sequencing. DNA was extracted according to a Power Soil DNA extraction kit (MoBio Laboratories, USA). For microbial community structure analysis, primers 341F and 805R were used to amplify the V3–V4 region as reported (Qu et al. 2016). The PCR products were loaded on Illumina-MiSeq device according to the manufacturer's protocols (Wu et al. 2019). After sequencing, data were collected and processed by Sangon Biotech Co., Ltd. The operational taxonomic unit (OTU) clustering was performed using Usearch (version 8.1.1831) at 97% sequence similarity threshold.

Results

Isolation and identification of degrading strains

After multiple transfers, the remaining PHs content was determined, and the microbial community with PHs degradation ability was selected to isolate single strain. In

the present study, 6 strains were isolated from LB solid medium, and only 2 strains (OBD-1 and OBD-3) showed obvious degradation ability (SFig. 1). Strain OBD-1 and strain OBD-3 could degrade 63% and 82% of PHs in 7 days, respectively. Strain OBD-3 had higher degradation effect than strain OBD-1 under the same conditions. Therefore, strain OBD-3 with higher degradation effect on PHs degradation was selected for identification.

The strain OBD-3 cultivated on LB solid medium showed a cheese colored, round, and glossy after cultivation for 3 days (SFig. 2a). The strain morphology of OBD-3 was rod-shaped (SFig. 2b). Based on alignment of the partial 16S rRNA gene sequence (Fig. 1a), the sequence of OBD-3 (GenBank accession No.: MW 404441) showed 100% identity with model strain *Rhodococcus qingshengii* djl-6 (Chuang et al. 2021), 99% identity with *Rhodococcus erythropolis* T7-2 (Huang et al. 2008), and 97% identity with model strain *Rhodococcus opacus* DSM 43205 ^{T}. On the other hand, the *gyrB* gene of OBD-3 revealed 100% identity with *Rhodococcus qingshengii* PT3-14, 99% identity with *Rhodococcus thermopolis* JCM 2892, and 87% with *Rhodococcus globerulus* ATCC 19370 (Fig. 1b). Thus, strain OBD-3 was *Rhodococcus qingshengii*, named *Rhodococcus* sp. OBD-3, which was stored in the China Center for Type Culture Collection (CCTCC M 2020978).

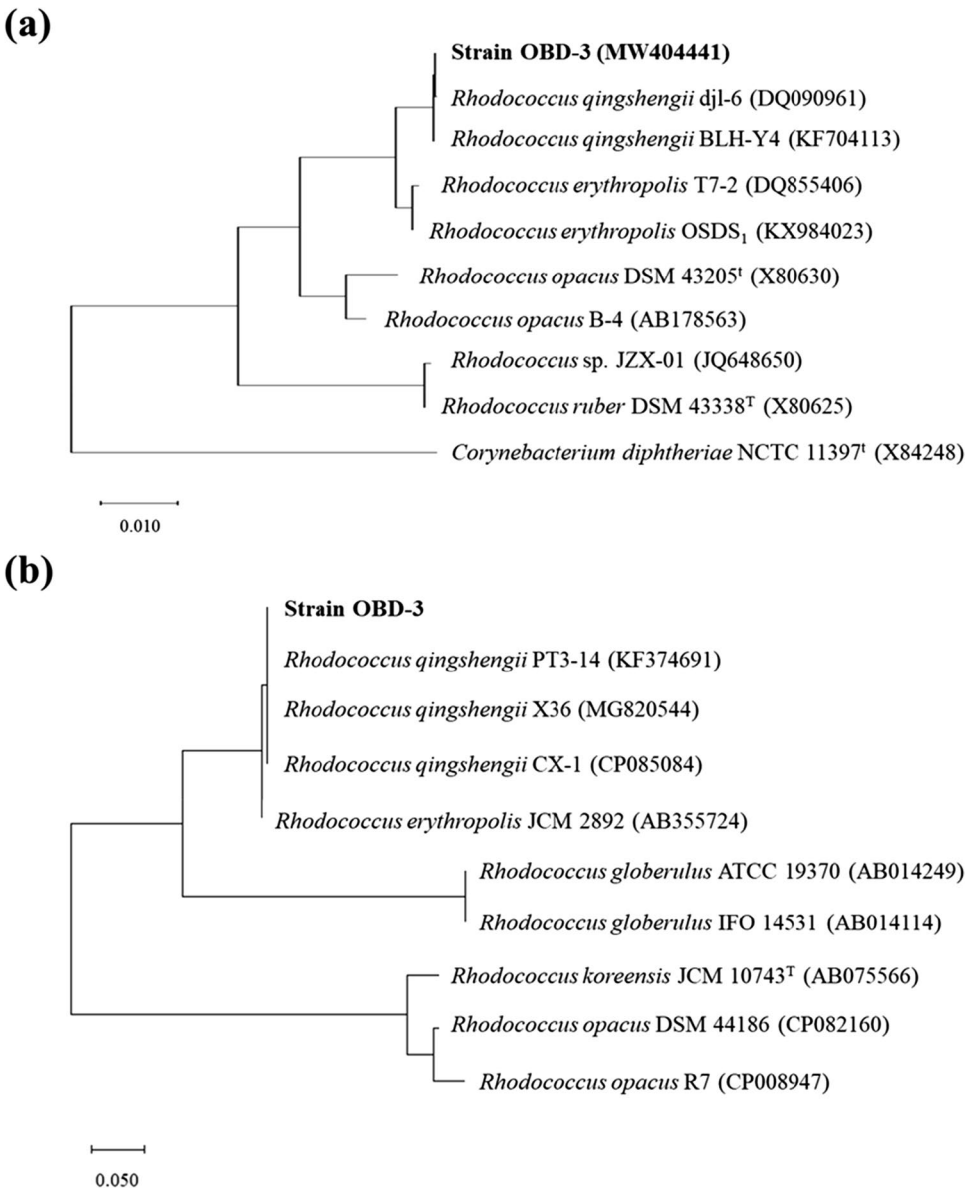


Fig. 1 Phylogenetic tree of strain OBD-3 based on the 16S rRNA gene and *gyrB* sequences. **a** 16S rRNA gene sequences analysis; **b** *gyrB* sequences analysis. The phylogenetic tree was determined by the neighbor-joining model. GenBank accession numbers were shown in parentheses

Degradation of PHs by strain OBD-3

The effect of conditions (pH, temperature, and salinity) on the biodegradation of PHs by strain OBD-3 was investigated as shown in Fig. 2. Strain OBD-3 biodegraded 75.3–86.6% of PHs at pH 6.0–9.0 (Fig. 2a), and more than 82.0% in neutral and alkaline environments (pH 7.0–9.0). Under different temperature conditions (Fig. 2b), PHs were degraded by more than 83.4% at 28–37 °C, and 60.6% ± 8.2% at low temperature (15 °C). In addition, strain OBD-3 has good degradation effect on PHs when the total salinity was 1–7% (Fig. 2c), which degraded 81.1–84.8%.

Under relatively optimal conditions (28 °C, pH 8.0, total salinity 1%), strain OBD-3 rapidly degraded 75.8 ± 4.7% of the PHs within 3 days, and 89.1 ± 1.8% in 7 days (Fig. 3c). During the degradation process, the contents of short-chain and medium-chain alkanes were significantly reduced (Fig. 3 a and b).

The detection of degradation genes in strain OBD-3

The *alkB1* and *alkB2* are the most common genes of alkane monooxygenase gene (Whyte et al. 2002), and their primers were used for PCR amplification with the OBD-3 genome as a template. The products were 634 bp and 500 bp, respectively (SFig. 3). Through the recovery and sequencing of products, the partial amino acid sequences were aligned (Fig. 4). Both AlkB1 and AlkB2 of strain OBD-3 have EHN(V)R(K)GHH and NYXEHYGL motifs that are highly conserved among all bacterial alkane monooxygenases (Whyte et al. 2002). The AlkB1 in strain OBD-3 showed 100% identity with AlkB1 from the low-temperature PHs degrading strain *Rhodococcus*

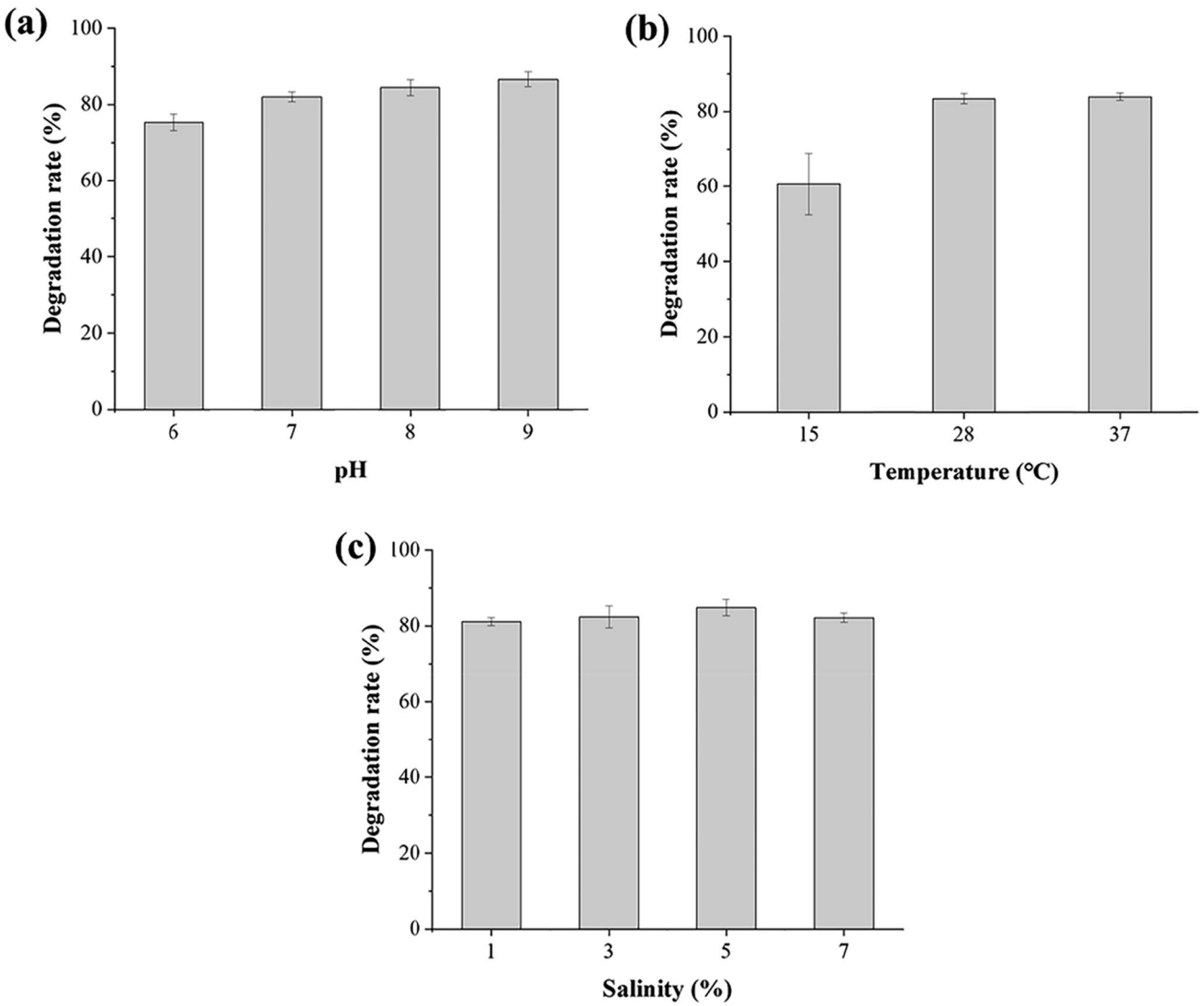


Fig. 2 The effects of growth conditions on PHs degradation by strain OBD-3. **a** pH; **b** temperature; **c** salinity

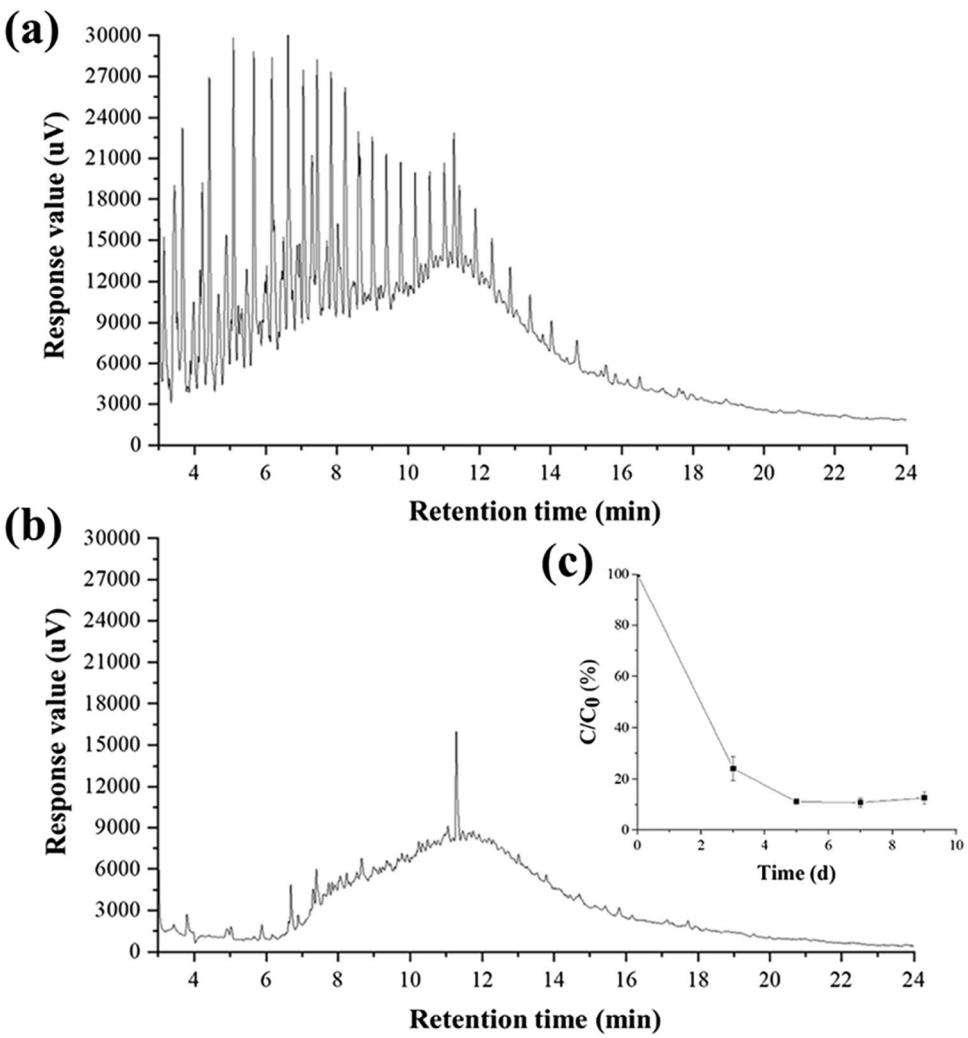


Fig. 3 The degradation of PHs by strain OBD-3. **a** GC profile of the undegraded sample; **b** GC profile of the degraded 7 days sample; **c** degradation curve

sp. TMP2 (Takei et al. 2008), 100% identity with AlkB1 from another low-temperature PHs degrading strain *Rhodococcus* sp. Q15 (Whyte et al. 2002), and 69% identity with AlkB2 in strain OBD-3 itself. The AlkB2 in strain OBD-3 revealed a 100% identity with AlkB2 from *Rhodococcus* sp. NRRL b-16531 and 99% identity with AlkB2 from *Rhodococcus* sp. Q15 (Whyte et al. 2002). AlkB1 and AlkB2 in strain OBD-3 were 50% and 50% identical to AlkB in the PHs degrading strain *Pseudomonas putida* GPo1 (Xie et al. 2011), respectively. Moreover, they showed 44–53% identity with AlkB1 and AlkB2 from *Pseudomonas aeruginosa* and *Alcanivorax borcumensis*. Therefore, AlkB1 and AlkB2 expressed by the *alkB1* (MZ688386) and *alkB2* (MZ688387) genes in strain OBD-3 all belonged to *Rhodococcus*.

The remediation of contaminated soil by strain OBD-3

The concentration of PHs in the test soil was 8556 ± 803 mg/kg, as determined by GC detection. After 2 months of remediation by strain OBD-3, the PHs in the soil were removed at 2049 ± 347 mg/kg in 1 month, and 2809 ± 597 mg/kg in 2 months. The microbial community structure in the soil also changed. The top 25 predominant genera in each sample and their relative abundances were shown in Fig. 5. In the initial contaminated soil (CK 0 M), unclassified *Gammaproteobacteria*, *Escherichia Shigella* and unclassified *Pseudonocardineae* were the dominant genera with relative abundances of 8.6%, 5.5%, and 5.1%, respectively. The relative abundances of *Sphingobium*, *Rhodococcus*, and *Pseudomonas* in the remediated soil (E 2 M) were 25.2%, 12.1%, and 10.3%, respectively. In addition, *Achromobacter* (0.27% in CK 0 M vs. 5.20% in the E 2 M), *Olivibacter* (0.01% in CK 0 M vs. 1.80% in the E 2 M), *Pandoraea* (0.02% in CK 0 M vs. 1.04% in the E 2 M), *Brevibacillus* (0.01% in CK 0 M vs. 0.62% in the E 2 M), and *Azospirillum* (0.03% in CK 0 M vs. 0.58% in the E 2 M) were significantly increased in relative abundance level of soil.

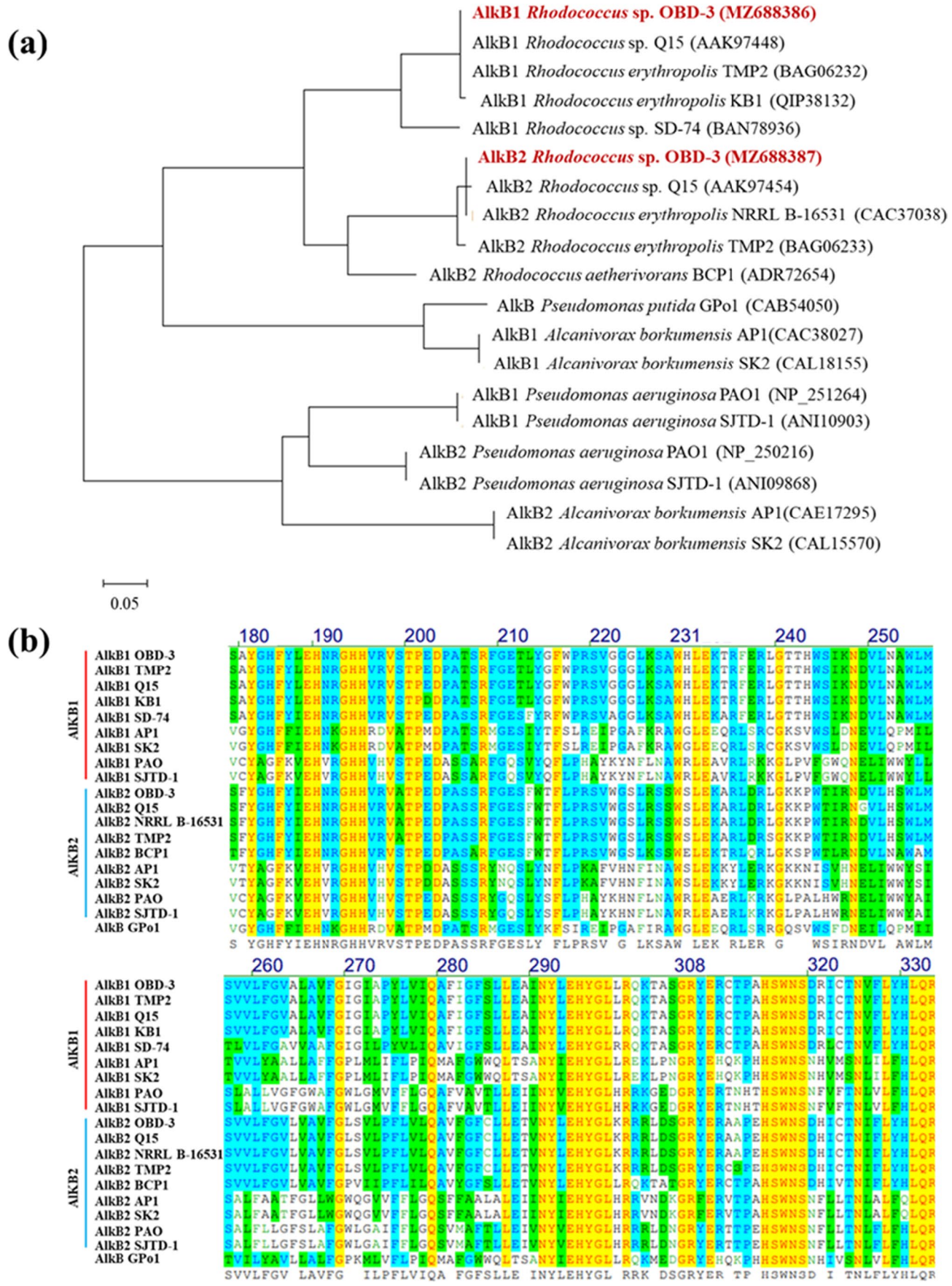
Discussion

In this study, the substrate (PHs) extracted from contaminated soil was used as the sole carbon source to isolate and identify high-efficiency degrading indigenous strains from the contaminated soil of oil depots. The reason for using the substrate extracted from the soil was to isolate indigenous degrading strains that were more suitable for the soil (Zhao et al. 2017). After the degrading strains were isolated, the degradation performance of the strains was studied by changing the pH, temperature, and salinity, and the degradation function genes of the strains were detected. The above process formed an isolation and identification system of indigenous degrading strains. In addition, the degrading strains were applied to the remediation of in situ contaminated soil, and the remediation effect of the strains was measured from the degradation effect and changes in microbial community structure. Finally, the isolation and application system of indigenous degrading strains was formed (Fig. 6).

Based on this system, the isolated strain OBD-3 was identified as *Rhodococcus*. *Rhodococcus* is a common hydrocarbons degrading bacterial genu that can persist and grow in highly contaminated soils and waters, and even under oxygen- and nutrient-limited conditions (Kuyukina and Ivshina 2010). *Rhodococcus* sp. HX-2 was a salt tolerant degrading strain screened from the oil field that could degrade more than 50% of 0.4% (v/v) diesel oil at 5% salinity (Hu et al. 2020). *Rhodococcus erythropolis* T7-2 was isolated from the oil-polluted sea-bed mud of Bohai Sea, which degraded diesel oil at 15 °C (Huang et al. 2008). *Rhodococcus* sp. JZX-01 decomposed 65.27 ± 5.63% of crude oil in 9 days, and had good oil degradation ability at low temperatures as well as under high salt conditions (Li et al. 2013). *Rhodococcus rubber* JC-106 could efficiently degrade crude oil at low temperature, which degraded 41.61% and 58.18% with crude oil as the sole carbon source at 15 °C and 35 °C for 15 days (Liu et al. 2016). In this study, strain OBD-3 had high degradation activity at 15 ℃, pH 9.0 and 7% salinity, and degraded most of PHs within 3 days. OBD-3 combined the characteristics of low-temperature resistance, saline alkali resistance, and rapid degradation in a short time. Compared with other *Rhodococcus*, OBD-3 has strong environmental adaptability, degradation ability, and potential application prospects.

Strain OBD-3 was more likely to degrade short-chain and medium-chain alkanes but not completely degraded PHs. Alkanes are most easily degraded by microorganisms due to their simple structure compared to other hydrocarbons (Verma et al. 2006). However, short-chain alkanes also have the potential for incomplete degradation due to their bioavailability and solubility in cell membranes, and substrate toxicity may be the reason for the limited biodegradation of long-chain alkanes reported by various studies (Lal and Khanna 1996; Deng et al. 2014). In the aerobic degradation of alkanes, alkanes were mainly catalyzed by alkane monooxygenases, which catalyzed terminal methylated carbon oxidation (Ji et al. 2013). The Alk system is one of the major alkane monooxygenases and the most studied hydrocarbon hydroxylation system. The Alk system in *Pseudomonas putida* GPo1 could oxidize C5–C12 *n*-alkanes to 1-alkanols, and catalyze a variety of reactions including the hydroxylation of linear and branched aliphatic, cycloaliphatic, and alkylaromatic compounds, branched demethylated methyl ethers and epoxidation of terminal olefins (van Beilen et al. 1994; van Beilen and Funhoff 2005). In *Alcanivorax borkumensis* SK2, there were two non-heme hydroxylases AlkB1 and AlkB2. AlkB1 preferentially hydroxylated C5–C12 hydrocarbons, while AlkB2 preferred to catalyze C8-C16 hydrocarbons (Hara et al. 2004). The *alkB1* and *alkB2* genes of *Rhodococcus opacus* B-4 were heterologously expressed in *E. coli* JM109, making JM109 degradable to C5-C16 alkanes (Sameshima et al. 2008). *Rhodococcus* sp. TMP2 contained five alkane-degrading monooxygenase genes *alkB1–alkB5*, which degraded straight-chain and branched alkanes from C9 to C24, but only the expression of *alkB1* and *alkB2* was induced by *n*-alkanes (Takei et al. 2008). Strain OBD-3 possessed *alkB1* and *alkB2* genes belonging to *Rhodococcus*. These genes played an important role in the degradation of alkanes, which was consistent with the characteristic that strain OBD-3 rapidly degraded short-chain and medium-chain alkanes.

In the bioremediation process, strain OBD-3 continuously degraded PHs in soil within 2 months. However, the remediation effect of OBD-3 was not particularly good, possibly due to the relatively high concentration of PHs in the soil and the lack of nutrient supplementation (Yuniati 2018). Except for the degradation of PHs, the microbial community structure also changed with the addition of OBD-3. *Rhodococcus*, *Sphingobium* and *Pseudomonas* became the dominant bacteria. The relative abundance of *Rhodococcus* was upregulated 388.5-fold compared with the initial contaminated soil. The other two dominant genera, *Sphingobium* and *Pseudomonas*, were upregulated 5.0-fold and 13.1-fold, respectively. These genera were frequently present in PHs-contaminated soils (Wang et al. 2020; Rodríguez-Uribe et al. 2021). *Sphingobium* was known for its natural ability to adjust to contaminated environments and use contaminants as a growth and energy source (Waigi et al. 2015). *Pseudomonas* was a common bacterium capable of degrading hydrocarbons, which could degrade crude oil, diesel oil and gasoline (Wongsa et al. 2004; Xie et al. 2011; Li et al. 2013). In addition, *Achromobacter* (upregulated 19-fold) and


(a)
AlkB1 Rhodococcus sp. OBD-3 (MZ688386)
AlkB1 Rhodococcus sp. Q15 (AAK97448)
AlkB1 Rhodococcus erythropolis TMP2 (BAG06232)
AlkB1 Rhodococcus erythropolis KB1 (QIP38132)
AlkB1 Rhodococcus sp. SD-74 (BAN78936)
AlkB2 Rhodococcus sp. OBD-3 (MZ688387)
AlkB2 Rhodococcus sp. Q15 (AAK97454)
AlkB2 Rhodococcus erythropolis NRRL B-16531 (CAC37038)
AlkB2 Rhodococcus erythropolis TMP2 (BAG06233)
AlkB2 Rhodococcus aetherivorans BCP1 (ADR72654)
AlkB Pseudomonas putida GPo1 (CAB54050)
AlkB1 Alcanivorax borkumensis AP1(CAC38027)
AlkB1 Alcanivorax borkumensis SK2 (CAL18155)
AlkB1 Pseudomonas aeruginosa PAO1 (NP_251264)
AlkB1 Pseudomonas aeruginosa SJTD-1 (ANI10903)
AlkB2 Pseudomonas aeruginosa PAO1 (NP_250216)
AlkB2 Pseudomonas aeruginosa SJTD-1 (ANI09868)
AlkB2 Alcanivorax borkumensis AP1(CAE17295)
AlkB2 Alcanivorax borkumensis SK2 (CAL15570)
0.05
(b)
180 190 200 210 220 231 240 250
AlkB1 OBD-3
AlkB1 TMP2
AlkB1 Q15
AlkB1 KB1
AlkB1 SD-74
AlkB1 AP1
AlkB1 SK2
AlkB1 PAO
AlkB1 SJTD-1
AlkB2 OBD-3
AlkB2 Q15
AlkB2 NRRL B-16531
AlkB2 TMP2
AlkB2 BCP1
AlkB2 AP1
AlkB2 SK2
AlkB2 PAO
AlkB2 SJTD-1
AlkB GPo1
S YGHFYIEHNRGHHVRVSTPEDPASSRFGESLY FLPRSV G LKSAW LEK RLER G WSIRNDVL AWLM
260 270 280 290 308 320 330
AlkB1
AlkB2
SVVLFGV LAVFG ILPFLVIQA FGFSLLE INYLEHYGL RRK DSGRYER TP HSWNSD I TNLFLYHLQR

◂**Fig. 4** Partial amino acid sequence analysis of alkane hydroxylases in strain OBD-3. **a** Phylogenetic tree with neighbor-joining model; **b** motif analysis of AlkB. GenBank accession numbers were shown in parentheses

Olivibacter (upregulated 138-fold), which did not account for a large proportion but increased by a large number, were also to degrade PHs (Deng et al. 2014; Szabo et al. 2011). In microbial communities that grew and degraded under PHs, the amplifiers that originated from the six major bacterial genera (*Pseudomonas*, *Sphingobium*, *Ochrobactrum*, *Achromobacter*, *Cupriavidus*, and *Parvibaculum*) accounted for more than 97% of the total amplifiers sequenced from diesel fuel cultures. Among them, *Pseudomonas* was the most important (relative abundance was 70.7%), followed by *Sphingobium* (12.3%), *Ochrobactrum* (6.1%), *Achromobacter* (4.6%), *Cupriavidus* (2.2%), *Parvibaculum* (1.1%), and *Olivibacter* (1.0%). This result was also similar to the

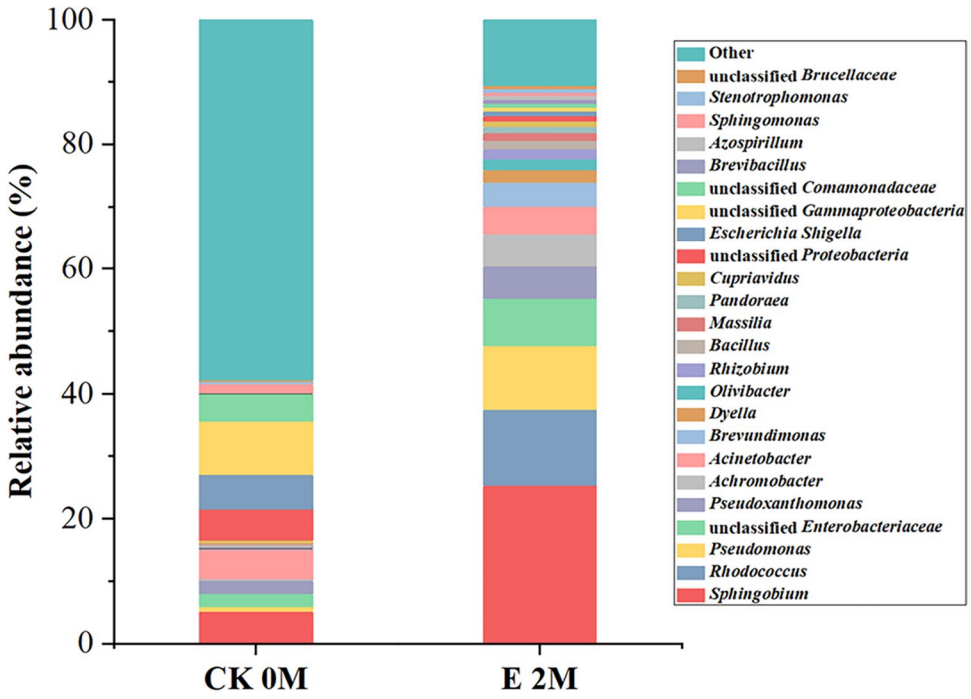


Fig. 5 Changes in microbial community structure at the genus level during soil remediation with strain OBD-3. The relative abundances in the plot were ordered the same as in the legend

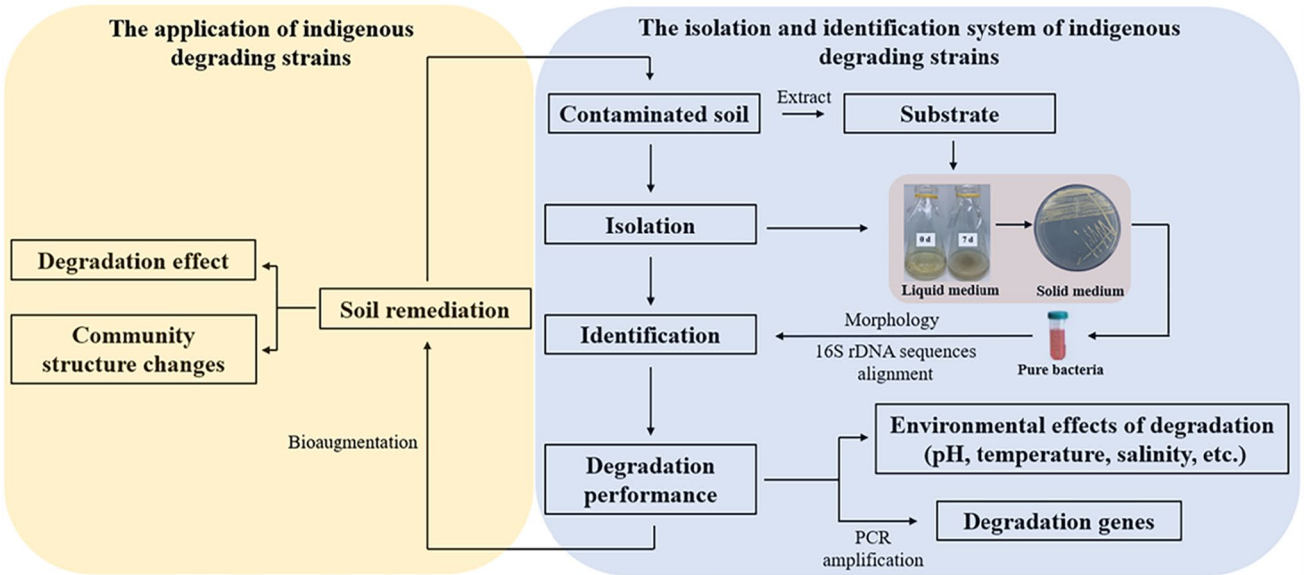


Fig. 6 The proposed routes for indigenous degrading strains from isolation to application

microbial community structure in soil after adding strain OBD-3. The addition of strain OBD-3 not only degraded PHs in the soil but also promoted the growth of other PHs degrading strain, to a obtain better remediation effect.

Conclusions

The indigenous PHs degrading strain *Rhodococcus* sp. OBD-3 was isolated and identified from the oil depot contaminated soil. It could degrade 60.6–86.6% of 1000 mg/L PHs within a broad range of temperatures (15–37 °C), pH (6.0–9.0), and salinities (1–7% NaCl), and could be efficiently degraded under extreme conditions (15 °C and 3–7% salinity), with good environmental adaptability. Strain OBD-3 contained alkane monooxygenase genes *alkB1* and *alkB2*, which belonged to *Rhodococcus*. An isolation and identification system of indigenous degrading strains was formed from the isolation of indigenous strains, the study of degradation performance to the detection of degradation genes. In addition, strain OBD-3 had a certain effect on the remediation of in situ contaminated soil, and increased the relative abundance of PHs degradation strains in soil. Strain OBD-3 had potential application prospects for the in situ bioremediation of PHs-contaminated soils.

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Author contribution CC and YL conceived the idea and designed the experiments. XC conducted the experiments, analyzed the data, and prepared first draft of the manuscript. JS, GS, FZ, and CC reviewed and revised the manuscript. All authors reviewed the manuscript.

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Data Availability Data available on request from the authors.

Declarations

Ethics approval and consent to participate This article does not contain any studies with human or animal subjects performed by any of the authors.

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Competing interests The authors declare no competing interests.

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