Screening and Preliminary Classification of High Efficiency Petroleum Hydrocarbon Degrading Bacteria

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Abstract: A strain Z-3 was screened from oil-polluted soil of Jilin Oilfield. Under the conditions of incubation for 7 days, initial concentration of petroleum 7.0 g/L, temperature 30 C and initial pH value of medium 7.0, the oil degradation rate can reach 50.6%. Bacillus cereus was initially identified by morphological observation, physiological and biochemical identification and 16S rDNA gene sequence analysis.

1. Introduction

With the rapid improvement of the national economy, the oil industry has developed rapidly, and the resulting oil pollution has become increasingly serious. At present, oil pollution treatment methods mainly include physical repair, chemical repair and bioremediation technologies [1]. Bioremediation is the process of adsorbing or degrading pollutants in the environment through biological metabolic processes. Among them, microbial remediation technology has become the most promising remediation technology for oil pollution treatment because of its good treatment effect, low cost, strong adaptability and no secondary pollution [2,3]. In this study, strains with good petroleum degradation ability were screened from oil-polluted soil of Jilin Oilfield, and the strains with the highest degradation rate were preliminarily classified and identified. In order to provide a theoretical basis for further research on culture conditions and optimization of degradation process.

2. Materials and methods

2.1 Test material

Strains: Oil contaminated soil samples from Jilin Oilfield. Petroleum: Dehydrated crude oil produced in Jilin Oilfield.

2.2 Culture medium

Inorganic salt medium: NaCl 11g, NH_4CL 0.7g, KH_2PO_4 0.5g, K_2HPO_4 1.2g, $MgSO_4$ 0.6g, $CaCl_2$ 0.03g, KCl 0.15g, $FeCl_2.4H_2O$ 0.02g, distilled water 1000mL.

LB liquid medium: peptone 10.0 g, yeast powder 5.0 g, NaCl 5.0 g, agar 15.0 g, distilled water 1000 mL, pH value to about 7.2.

2.3 Experimental method

2.3.1 Screening of Strains

The collected oil field soil samples were added to LB liquid culture medium, enriched and cultured in shaking bed at 30 C for 7 days, then single colonies were separated and selected by gradient dilution method. Single colonies of different colors and shapes were cultured and stored in LB solid culture medium. The strain was inoculated into MSM medium containing petroleum and cultured at 31℃ on a shaker to examine the ability of the strain to degrade petroleum.

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2.3.2 Physiological and biochemical identification of strains

The strains with higher degradation ability were numbered, and the growth state, colony characteristics and bacterial morphology of the strains were observed. Gram staining, spore staining, semi-solid test, nitrate reduction test, citrate utilization test, glucose fermentation test and other physiological and biochemical identification were carried out.

2.3.3 16S rDNA Sequencing of Strains

Extraction of genomic DNA

Single colonies were selected and inoculated into a triangular flask containing 20 mL LB medium for 160 r/min and incubated at 30 ~C for 24 hours. Take 1 mL culture medium into 2 mL Eppendorf tube, centrifuge 8000 rpm for 2.5 minutes, then pour out the supernatant. Add 130 μL of TE to break up the bacteria and add 10 μl/mL of lysozyme 70 μL. Allow to stand at 37 ° C for 10 min. Add 500 μL of Digestion Buffer and mix gently. Add 5 μL of Protein K, mix gently, and incubate at 50 °C for 7 min.

Add 300 μL of ethanol, mix and transfer to the UNIQ-10 column. Centrifuge at 10,000 rpm for 1 min and pour off the liquid in the collection tube. Add 450 μL of 70% ethanol and centrifuge at 10,000 rpm for 0.5 min. Repeat the above procedure. The ethanol was thoroughly dried by centrifugation at 10,000 rpm for 2 minutes. The adsorption column was transferred to a new 1.5 mL centrifugal tube. The elution buffer was preheated at 60 C for 50_L and placed at room temperature for 3 minutes. 12 000 rpm centrifugation for 2 minutes, the liquid flow is genomic DNA. The above liquid electrophoresis was used to detect the quality.

PCR amplification

Conservative amplification primers were designed based on published 16S rDNA sequences.

7(F) 5'- CAGAGTTTGATCCTGGCT -3'

1540 (R) 5'- AGGAGGTGATCCAGCCGCA -3'

In the 0.2 mL Eppendorf tube, the genomic DNA (20-50ng/mu L) of the strain to be identified was extracted by adding 0.5 mu L, followed by the following reaction mixture:

7(F) (10μM)	0.5μL
1540(R) (10μM)	0.5μL
10×PCR Buffer	2.5μL
dNTP (2.5 UL each)	1μL
Taq enzyme	0.2 μL

Add ddH2O to adjust the reaction system to 25 μL, and mix by simple centrifugation.

PCR reaction

Place the Eppendorf tube into the PCR machine and cover the lid. The amplification conditions are:

94℃	4 min	
94℃	45 sec	30 cycles
55℃	45 sec	
72℃	1 min	
72℃	10 min	

The reaction was stopped at 4℃ and the product was stored at 4℃.

Detection of PCR products by electrophoresis

Take out the Eppendorf tube, take out the reaction product of 10 UG L, add 2 UG L sample buffer, gently mix. In the 1% prepared agarose gel, the first step was to enter 10000bpmaker at a point and then to PCR product. The electrophoresis time was 20min at 150V and 100mA conditions. The gel was removed with a disposable glove, stained with EB for 30 min, and the amplification results were observed in a gel imager.

Recovery of amplified fragments

If the amplification product described above is a unique band, the amplification product can be directly recovered. Otherwise the target nucleic acid band is cleaved from the agarose gel.

DNA fragment sequencing

The samples were sequenced by Shanghai Shenggong.

3. Results and analysis

3.1 Screening results of strains

After enrichment and domestication, three strains of oil-degrading bacteria were obtained, numbered Z-1 Z-3, respectively. The oil degradation ability of each strain is shown in Table 1. It can be seen from Table 1 that the Z-3 strain has the strongest degradation ability, and the Z-3 strain was selected as the research object in the subsequent experiments.

Table 1 Comparison of oil degradation ability of strains

Strain	Z-1	Z-2	Z-3
Degradation rate /%	40.2	35.4	50.6

3.2 Physiological and Biochemical Identification of Strain Z-3

The single colony formed by strain Z-3 was round, yellow-white and flat, and its surface was waxy and prone to provocation. The strain is Gram-positive bacilli with spores and flagella. Bacterial cells are rod-shaped, forming short or long chains and producing red pigments after long storage. A photograph of the strain Z-3 is shown in Fig. 1, and the results of the physiological and biochemical reaction measurement are shown in Table 2.

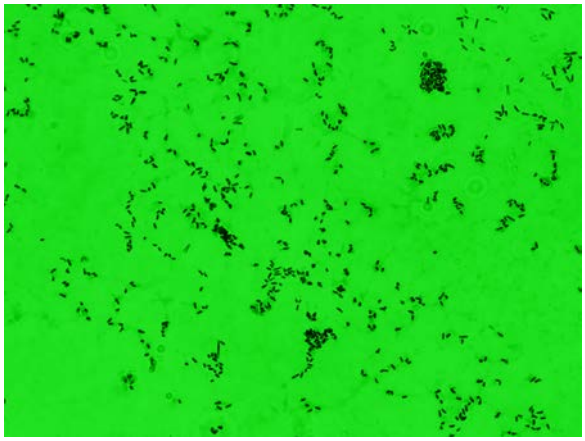

Fig.1. Z-3 photo

Table 2 Experimental results of physiological and biochemical reactions of strain Z-3

Test item	Gram staining	Spore dyeing	Semisolid	Nitrate reduction	Utilization of citrate	Glucose Fermentation			
Test result	+	+	+	+	+	+			
Test item	Starch hydrolysis	Contact enzyme	Gelatin	Tyrosine	Xylose fermentation	Mannose Fermentation	Aerobic	V-P	MR
Test result	+	+	+	+	-	-	-	+	+

+ Positive reaction; - negative reaction.

From Table 2, we can see that Z-3 strain is similar to Bacillus microorganisms. Tyrosine test of Z-3 strain is positive, which is a typical feature of Bacillus cereus.

3.3 16S rDNA Sequencing Results of Strains

1) PCR amplification results

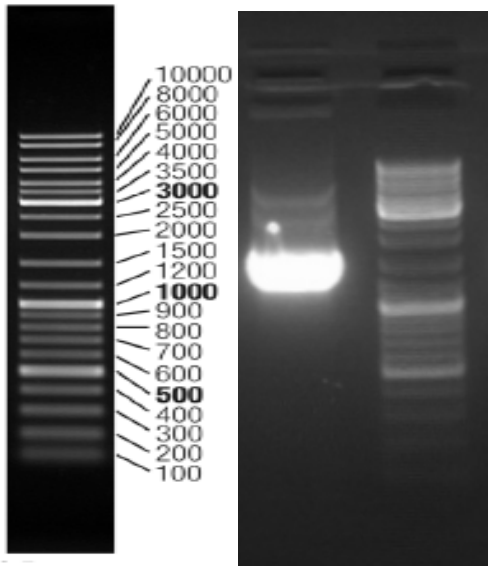

Fig.2. PCR amplification results of strain Z-3

2) PCR amplified fragment sequencing results

3) 1455bp

CCGGGTTTCTTAATACATGCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAG
TTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCC
GGGAAACCGGGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGC
GGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACG
GCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACT
GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGA
AAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGA
AAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCC
GGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCAC
GGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGT
GGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGG
CGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATT
AGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCC
CTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTG
AAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA
AGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCT
TCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGA
TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGG
GCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCA
TCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCA
AGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAA
CTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACG
TTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCG
GTGGGGTAACCTTTTTGGAGCCAGCCGCCTAAGGTGGACAGGATT

D. Sequencing results analysis

- domain Bacteria (0/20/1408156)
- phylum Firmicutes (0/20/459584)
- class Bacilli (0/20/278921)
- order Bacillales (0/20/178527)
- family Bacillaceae 1 (0/20/38457)
- genus Bacillus (0/20/29955)

S000001726 not_calculated 1.000 1188 Bacillus sp. 240B1; AF350926

S000372240	not_calculated	1.000	1340	Bacillus cereus; Y1; AY651924
S000625383	not_calculated	1.000	1348	Bacillus thuringiensis; DQ286303
S000625385	not_calculated	1.000	1353	Bacillus thuringiensis; DQ286305
S000625402	not_calculated	1.000	1340	Bacillus thuringiensis; DQ286322
S000625412	not_calculated	1.000	1339	Bacillus thuringiensis; DQ286332
S000625437	not_calculated	1.000	1344	Bacillus thuringiensis; DQ286357

The 16S rDNA sequence of Z-3 strain was compared with the GenBank database, and the sequence alignment of the 16S rDNA sequences of similar bacteria was obtained. The homology of the 16S rDNA sequence of the strain was 99% (see Figure 3). According to the results of microbial physiological and biochemical characteristics (Table 2), strain Z-3 was identified as Bacillus cereus.

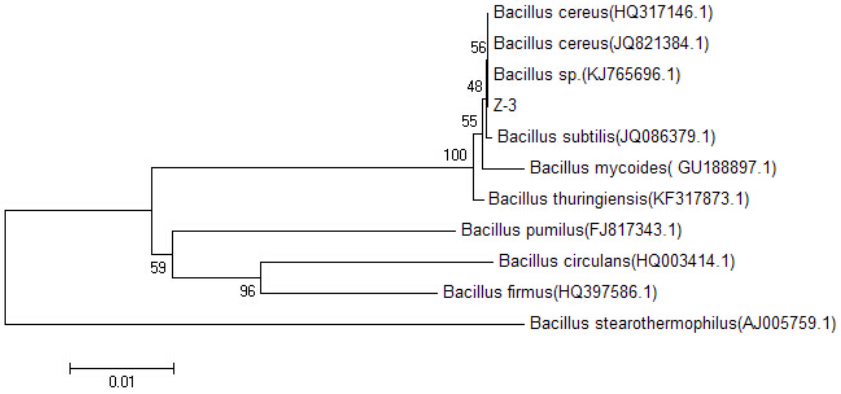

Fig.3. Phylogenetic tree of strain Z-3

4. Conclusion

In this study, soil samples were taken from Jilin Oilfield, and 3 strains with petroleum degradation ability were obtained from the selection, numbered Z-1 Z-3. Under the conditions of 7d culture time, petroleum concentration 7.0 g/L, temperature 30 °C, and initial pH value of 7.0, the degradation rates of Z-1 Z-3 strains were 40.2%, 35.4% and 50.6, respectively. %, Z-3 strain has the best degradation performance. The morphological characteristics and culture characteristics of the bacteria were observed, and the physiological and biochemical identification and 16S rDNA gene sequence analysis were carried out. The single colony of strain Z-3 was round, yellow-white, flat and waxy. The strain was Gram-positive bacillus with spores and flagella. The 16S rDNA sequence of Bacillus cereus was 99% homologous with that of Bacillus cereus, and was preliminarily identified as Bacillus cereus. The strain has the value of continuing research. In the later stage, the study of culture parameters, optimization of degradation conditions and development of compound microbial agents will be carried out.

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References

[1] Gong H, Bao M , Pi G , et al. Dodecanol-Modified Petroleum Hydrocarbon Degrading Bacteria for Oil Spill Remediation: Double Effect on Dispersion and Degradation[J]. ACS Sustainable Chemistry & Engineering, 2016, 4(1):169-176.

[2] Guo P , Lin J G , Cao B X , et al. Isolation of Petroleum Hydrocarbon Degrading Bacteria from the Liaohe Estuary at Cold Climate[J]. Advanced Materials Research, 2015, 1092-1093(48):4.

[3] Raju M N , Masabanda M , Leiva-Mora M . Novel diesel-oil-degrading bacteria and fungi from the Ecuadorian Amazon rainforest[J]. Water Science & Technology, 2015, 71(10):1554-1561.