Study on the Influence Mechanism of Chitosan on Two Strains of Petroleum Hydrocarbon Degrading Bacteria

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Abstract: With the rapid increase of petroleum use in modern industrialization, the leakage in the process of exploitation and transportation has caused environmental issue. In order to degrade the pollutants in a green way, it is very important to study the properties of petroleum hydrocarbon degrading bacteria. The effect of promising immobilized microbial material, chitosan, on the hydrocarbon degrading bacteria was studied by plate drilling method. It was found that the acetic acid used to dissolve chitosan had a certain inhibitory effect on the growth and development of microorganisms. Chitosan had the ability to resist such inhibitory effect and this ability also depended on the strain itself to a certain extent.

1 INTRODUCTION

With the accelerating process of industrialization and the increasing demand for petroleum and its ancillary products, petroleum, known as the "blood" of modern industry, will inevitably have a negative impact on society from exploitation to use (Dai. 2014). The average annual output of oil in the world is about 4 billion tons. According to the statistics, 2 kg of pollutants enter the environment every 1 t of oil produced, so about 800 wt of pollutants enter the environment every year in the world (Wei. 2020).

At present, there are a lot of remediation technologies for oil pollution, and bioremediation technology has great application potential because of its safety, environmental protection, no secondary pollution and other advantages. Compared with the use of free cells, immobilized microorganisms have several advantages. Chitosan, a new functional carrier material, is the only basic polysaccharide found in nature so far. It is the product of chitin deacetylation. Its chemical name is polyglucosamine (1-4)-2-amino-b-d-glucose. Its good biocompatibility, strong adsorption and other excellent properties have been widely studied in textile printing and dyeing, food, papermaking, heavy metal recovery, medical and pharmaceutical, water treatment and other fields research (Gao et al. 2020; Ou et al. 2020). Chitin waste generated by shrimp culture can be recycled, which is a profitable source of income in areas with relatively developed aquaculture industry (Barreto et al. 2010).

CTS molecular surface is rich in functional groups but can only dissolve in weak acids. It is reported that in acidic environment, it can adsorb organic matter but its $-NH_2$ protonation into positively charged $-NH^{3+}$ will produce certain inhibitory effect on microorganisms. Therefore, for chitosan, the material used by immobilized microorganisms for degrading petroleum hydrocarbons, we should not only stay in the study of pollutants but also pay attention to its effects on different petroleum degrading microorganisms. In this paper, the effects of different concentrations of CTS on different petroleum hydrocarbon degrading bacteria were compared to provide a theoretical basis for the petroleum hydrocarbon degrading in the soil environment.

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2 MANUSCRIPT PREPARATION

2.1 Materials

The soil samples used in this study were obtained from the soil that was not contaminated by oil in Dongying, China. All other chemicals were of analytical grade and commercially available. The chitosan solution is prepared by dissolving powder CTS in different concentration of HAC.

2.2 Bacterial Strains and Culture Media

Mineral salt medium was used for sieve bacteria, the composition was as follows: 1.0 g of $(NH_4)_2SO_4$, 1.0 g of K_2HPO_4, 1.0 g of KH_2PO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.02 g of $CaCl_2$, 10.0 g of NaCl, 0.3 g of Crude oil in per liter of deionized water. Beef extract peptone liquid medium was used for inocula preparation and maintenance, and the composition was as follows: 5.0 g of NaCl, 5.0 g of beef extract, 10.0 g of tryptone in per liter of deionized water. Adjust pH to 7. Sterilization for 20 min at 121 ℃. After 4 weeks of domestication, absorb 1.0 mL of the supernatant and placed it in a glycerin tube and stored in the refrigerator for subsequent use.

2.3 Analytical Techniques

The DNA of strain was extracted from the high efficient petroleum hydrocarbon degrading bacteria by using universal primers 27f and 1492r. PCR amplification of 16S rRNA gene was carried out according to the corresponding system and procedure. PCR products were sequenced by Beijing Qingke Biotechnology Co., Ltd. According to the results of homology comparison, the genus was preliminarily identified.

2.4 Experimental Design

The CTS was dissolved in 2.0% and 1.0%HAC to prepare CTS solution with the concentration of 0.1%, 0.25%, 0.5%, 0.75%, 1.0% and 1.5%. The solution was mixed uniformly by ultrasound and sterilized at 105 ℃. Add 200 μL of target bacteria into 20 mL beef agar medium to be solidified, mix well, and make a plate. After the plate was solidified, three uniform holes were made on the plate with a 6.5 mm diameter punch as parallel holes. 50 μL CTS solution with different concentrations was injected into the holes, and 1.0% and 2.0% HAC was injected into the holes as control. The diameter of transparent circle was measured after 24 h cultivation at 37 ℃.

3 TEST RESULTS AND DISCUSSIONS

3.1 Experimental Results

Six strains of bacteria were screened from oil contaminated soil, and the oil reduction rate of each strain was determined. Two different strains were selected, one was *Pseudomonas aeruginosa* (TL-1) and the other was *Paenibacillus* (YB-1). The phylogenetic tree based on 16S rRNA sequence is shown in Figure 1.

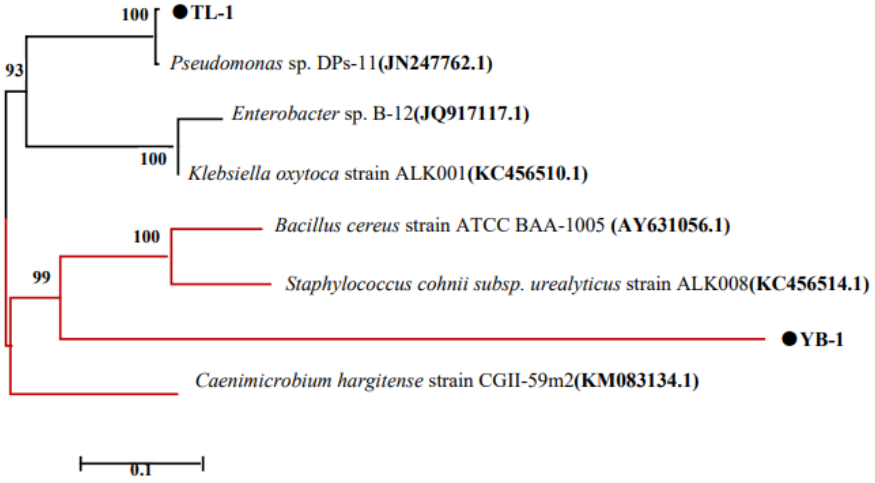

Figure 1: Phylogenetic tree based on 16S rRNA sequence.

3.2 Effect of Chitosan on Bacteria

The transparent circle formed by different concentrations of CTS dissolved in 1% HAC on the plate of strain YB-1 and TL-1 is shown in Fig. 3. For strain TL-1, the inhibition zone was the largest when the CTS addition was zero (1% HAC solution control group), and then with the increase of CTS concentration, the inhibition zone gradually decreased to the minimum when the CTS concentration was 1.5%.

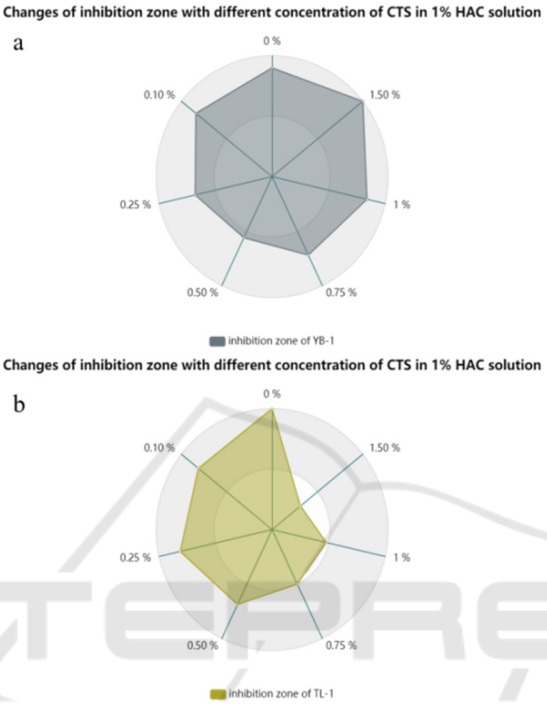

Figure 2: Inhibition zone of YB-1 and TL-1 in different concentration of CTS and 1% HAC.

For strain YB-1, 1% HAC alone made the inhibition zone larger, then with the continuous addition of CTS, the inhibition zone gradually decreased to the minimum when the concentration of CTS was 0.5%. After that, the inhibition zone increased gradually with the addition of CTS, and there is a trend that the inhibition zone will be larger than that formed when 1% HAC solution was injected alone. We speculate that when the concentration of CTS was less than 0.5%, the effect of HAC on the strain was dominant and the addition of CTS could gradually weaken the effect of HAC on the strain. But, with the gradual progress of the reaction, the $-NH_2$ on the surface of CTS in HAC is gradually protonated into positively charged $-NH^{3+}$, which makes CTS become a water-soluble cationic polyelectrolyte.

Variation of inhibition zone of strain YB-1 with CTS dissolved in different concentrations of HAC is as shown in Fig. 3. As for YB-1, the inhibition zone in CTS dissolved in 2% HAC is higher than that in 1% HAC. It can be concluded that HAC is the main inhibitor in this concentration range, and with the increase of CTS, not only does it not have a stronger antibacterial effect, but it can weaken the inhibitory effect of HAC on strain TL-1.

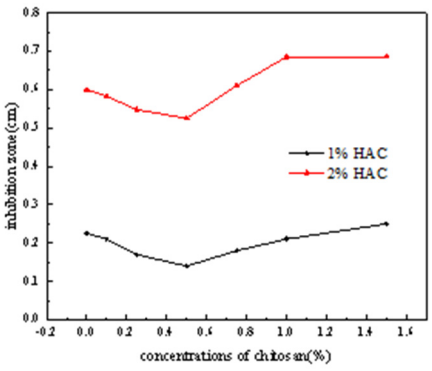

Figure 3: Variation of inhibition zone of strain YB-1 with CTS dissolved in different concentrations of HAC.

At the same time, the inhibition zone produced by two strains at different concentrations of CTS dissolved in 1% HAC was compared, as shown in Fig. 4.

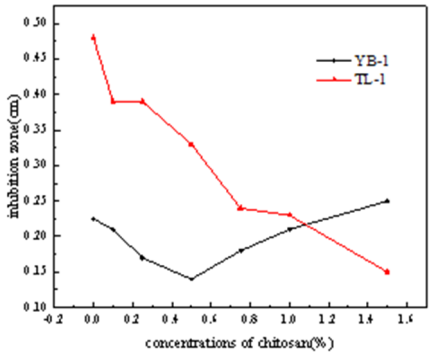

Figure 4: the inhibition zone of two strains under different concentrations of chitosan dissolved in 1% HAC.

When the concentration of CTS was less than 1%, the inhibition zone around strain YB-1 was significantly smaller than that of strain TL-1. The reason why we got this result is probably because YB-1 is a Bacillus. The spores produced by YB-1 can produce certain resistance to the external environment, so it can resist the influence of HAC on the strain, and the inhibition zone is much smaller than that of Gram-negative bacteria TL-1. So the inhibition of HAC on the strain also depends on the strain itself. Jing Yingjun (Jing et al. 2006) also pointed out that different molecular weight, pH value, metal ions and other external factors of CTS have different antibacterial effects on different strains, but the key factor of antibacterial activity of CTS is the strain itself.

4 CONCLUSIONS

In this paper, plate drilling method is adopted to study the effect of chitosan material on petroleum hydrocarbon degrading bacteria. The main conclusions can be summarized as follows:

(1) Two representative petroleum hydrocarbon degrading bacteria *Pseudomonas Aeruginosa* and *Paenibacillus* were successfully screened out from oil-contaminated soil.

(2) The main inhibitory factor on strain was acetic acid, chitosan can counteract this inhibitory effect under certain conditions.

(3) The inhibitory effect of HAC and CTS on the strain also depended on the strain itself.

(4) In terms of the future work, how to prepare immobilized microbial materials by dissolving chitosan under appropriate conditions to remove petroleum pollutants should be carried out.

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REFERENCES

Barreto, R..V.G., Hissa, D.C., Paes, F.A., Grangeiro, T.B., Nascimento, R.F.,Rebelo, L.M., Craveiro, A.A., and Melo, V. M. M. 2010. New approach for petroleum hydrocarbon degradation using bacterial spores entrapped in chitosan beads. Bioresource Technology 101(7):2121–25.

Dai, G. (2014). Analysis of the effect of different economic management modes on the oil industry. Economic perspective 20:401.

Gao,X., Z. Li, J. Gong., Q. Li, F. Li, and J. Zhang. 2020. Research progress on new bio-antibacterial agents for textiles. Journal of Textile Research 41(02): 187-92.

Jing, Y., Y. Hao, H. Qu, Y. Shan, D. Li and R. Du. 2006. Preliminary studies on antibacterial mechanism and analysis of antibacterial activity of chitosans. Chinese Journal of Antibiotics 31(6):361-65. doi: 10.13461 /j.cnki .cja.003748.

Liu, H., Y. Du, and X. Wang. Liping Sun Chitosan kills bacteria through cell membrane damage. Internationnal Journal of Food Microbiology 95(2): 147-55.

OY, X., Sheng, Y. Wang, Y. Lin, W. Chen and Y. Shi. 2020. Preparation of Biodegradable Chitosan Composite Film and Its Fresh-keeping Application in Chicken Breast. Food Research and Development 41 (21): 123-28.

Wei, Y. (2020). The Harm and Present Situation of Soil Oil Pollution. China Resources Comprehensive Utilization 38(04):120-122.