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Fungal diversity in major oil-shale mines in China

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ABSTRACT

As an insufficiently utilized energy resource, oil shale is conducive to the formation of characteristic microbial communities due to its special geological origins. However, little is known about fungal diversity in oil shale. Polymerase chain reaction cloning was used to construct the fungal ribosomal deoxyribonucleic acid internal transcribed spacer (rDNA ITS) clone libraries of Huadian Mine in Jilin Province, Maoming Mine in Guangdong Province, and Fushun Mine in Liaoning Province. Pure culture and molecular identification were applied for the isolation of cultivable fungi in fresh oil shale of each mine. Results of clone libraries indicated that each mine had over 50% Ascomycota (58.4%–98.9%) and 1.1%–13.5% unidentified fungi. Fushun Mine and Huadian Mine had 5.9% and 28.1% Basidiomycota, respectively. Huadian Mine showed the highest fungal diversity, followed by Fushun Mine and Maoming Mine. Jaccard indexes showed that the similarities between any two of three fungal communities at the genus level were very low, indicating that fungi in each mine developed independently during the long geological adaptation and formed a community composition fitting the environment. In the fresh oil-shale samples of the three mines, cultivable fungal phyla were consistent with the results of clone libraries. Fifteen genera and several unidentified fungi were identified as Ascomycota and Basidiomycota using pure culture. *Penicillium* was the only genus found in all three mines. These findings contributed to gaining a clear understanding of current fungal resources in major oil-shale mines in China and provided useful information for relevant studies on isolation of indigenous fungi carrying functional genes from oil shale.

Introduction

Oil shale is a type of fine-grained sedimentary rock that contains organic matter. Components of organic matter trapped in oil shale can yield significant amounts of oil and combustible gas when they undergo destructive distillation (Dyni, 2003). With the globally rising demand for energy and the gradual exhaustion of conventional energy resources such as petroleum, natural gas, and coals, oil shale is seen as an important replacement energy in the 21st century, considering its abundant reserves, advantageous features, and feasibility of exploitation (Bunger et al., 2004).

In the past, oil shale was mainly utilized with physical and chemical techniques. However, in recent years, the advantages of microbial metallurgy have been highlighted, such as simple technical processing, low-carbon and environmentally friendly operation, low energy consumption, and low costs (Anjum et al., 2012). In early studies, most inorganic matter in oil shale was directly or indirectly biodegraded by microorganisms under mild conditions, and kerogens remained as energy sources (Findley et al., 1974; Craig Meyer and Yen, 1976; Vrvić et al., 1988, 1990; Cvetković et al., 1993; Beškoski et al., 2008). Until 1997, organic matter in oil shale was found to be the primary energy source for

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indigenous bacteria (Krumholtz et al., 1997). Subsequently, oil shale was confirmed to provide the sole carbon source and energy for the growth of individual or mixed bacterial strains accompanying structural changes of oil shale (Permanyer et al., 2010; Matlakowska and Sklodowska, 2009; Matlakowska et al., 2010). In addition, mixed strains (*Rhodococcus erythropolis* and *Rhodococcus ruber*) that produce biosurfactants were used to degrade the organic matter in oil shale, indicating that the maximum extraction ratio of shale oil was as high as 26% (Haddadin et al., 2009). According to existing literature, bacteria play dominant roles in oil-shale bio-utilization. However, studies on fungi in oil shale are limited. In general, fungi are involved in almost all of the biological and biochemical reactions in the environment. Early studies found that some fungi can live on the surface of oil-shale particles in oil-shale deposits of the eastern US; however, further studies were not carried out (Pfister et al., 1991). In 2006, the known wood-rotting fungus *Schizophyllum commune* was applied for the biotransformation of oil shale, proving that the organic matter in oil shale can be decomposed and released to the surrounding environment as organic heavy metal complexes (Wengel et al., 2006). Although this study showed the potential of fungi for oil-shale bio-utilization, the strain used was not isolated from the habitat of oil shale. Indigenous microorganisms are capable of developing functional genes and special physiological metabolisms fitting the surrounding environment in the long-term adaptation process, which make them exhibit more proficient bio-utilization abilities for materials in their environment of origin (Qi et al., 2011; Matlakowska and Sklodowska, 2011). Therefore, exploring valuable indigenous fungi is an important and feasible way to propel the bio-utilization of oil shale. A survey on fungal diversity in the original environment is a fundamental prerequisite because it can provide useful information for targeted and expanded screening of valuable indigenous fungi.

Although China has abundant oil-shale resources that rank No. 4 in the world, available research and information on the microbial resources in oil shale are limited. In this study, three main oil-shale production areas in China were selected, including Huadian Mine in Jilin Province, Maoming Mine in Guangdong Province, and Fushun Mine in Liaoning Province. By combining the clone libraries of ribosomal deoxyribonucleic acid internal transcribed spacer (rDNA ITS) with traditional pure culture, the composition and structure, dominant group and diversity of fungus communities in the special habitats were revealed in detail. This study was aimed at providing a useful basis of fungal resources for the bio-utilization of oil shale and bioremediation of the ecological environment in oil-shale areas.

1. Materials and methods

1.1. Study plot setting and sample collection

The sampling was carried out in July 2012. In Maoming Mine in Guangdong Province (21°41′N, 110°58′E), Fushun Mine in Liaoning Province (41°50′N, 123°57′E), and Huadian Mine in Jilin Province (43°00′N, 126°47′E), the most representative fresh oil shale, weathered oil shale, and sandy soil were collected. The samples from the same mine were equally mixed for microbial total DNA extraction to construct the clone library. Fresh oil-shale samples were used for pure culture.

The samples in one mine were taken as examples. For each sample, three sampling plots with size of 10 m × 10 m and spacing of about 500–1000 m were set in each sampling area. The multi-point sampling method was used in each sampling plot. First, the surface shale or sandy soil was removed to avoid microorganisms in the external environment entering into samples. Next, crushed samples were collected with sterile gloves. After mixing, samples were filtered with a 4 mm sieve. An adequate amount of each sample was placed in 50 mL sterile centrifuge tubes by the quartering approach. Subsequently, the samples were brought back in an icebox and preserved in the laboratory at 4 °C. All sampling tools were autoclaved. The climatic characteristics of each mine are listed in Table 1, and the geological characteristics of each mine are listed in Table 2. The basic features of samples are listed in Tables 3 and 4.

1.2. Extraction of microbial total DNA

The improved sodium dodecyl sulfate SDS-high-salt extraction method was used based on Zhou's method (Zhou et al., 1996). For the specific operation procedures and the productivity and purity of extracted DNA, please refer to our previous publication (Jiang et al., 2014).

1.3. Clone libraries of fungal rDNA ITS

1.3.1. Construction of clone libraries

With ITS1: 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4: 5′-TCCTCCGCTTATTGATATGC-3′ as primers (Gardes and Bruns, 1993), 550 bp of rDNA ITS was amplified. The 50 μL PCR system consisted of 25 μL of 2 × MasterMix (Bioteke, China), 50 ng of microbial total DNA, 2 μL of each primer (10 μmol/L), and double distilled water. PCR amplification was performed on a C1000™ Thermal Cycler (Bio-Rad, USA) under the following conditions: initial denaturation at 94 °C for 5 min followed by 35 cycles of 94 °C denaturation for 30 sec, 55 °C annealing for 30 sec, 72 °C elongation for 45 sec, and a final elongation step at 72 °C for 10 min. PCR products were recycled and purified with the MiniBEST DNA Fragment Purification Kit (TaKaRa, China). The PCR product was ligated into the pUM-T vector (Bioteke, China), and the ligation reaction was used to transform competent *Escherichia coli* strain DH5α (Bioteke, China) to generate rDNA ITS clones. Recombinant clones were selected on Luria-Bertani agar plates containing 20 μg/mL X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), 0.5 mmol/L IPTG (isopropyl-β-D-thiogalactopyranoside), and 100 μg/mL ampicillin. Plates were incubated overnight at 37 °C. The presence of inserts was determined by PCR with white (positive) bacterial colonies by using primers M13F: 5′-GTAAAACGACGGCCAG-3′ and M13R: 5′-CAGGAAACAGCTATGACCATG-3′.

Primer synthesis and sequencing of positive clones were completed by Sangon Biotech (Shanghai, China) Co., Ltd. Chimeras were detected online by the Bellerophon system. DNAman software was used to remove vector sequences, and the VecScreen system was used to examine whether the