Supplementary Materials

for

Combined application of resveratrol and a ryegrass endophyte in PAH-contaminated soil remediation and its impact on soil microbial communities

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M1: Isolation and identification of the PAH-degrading bacteria

A: *Methylobacterium extorquens* C1

The root of ryegrass samples were cut and ground with a pestle in a mortar containing 10 mL of sterile distilled water to obtain root suspensions. The supernatant solution (3.0 mL) was added to a 250-mL Erlenmeyer flask containing 100 mL of PMM and maintained for 72 h at 30±1℃ and 120 rpm in an orbital shaker. Then, a series of dilutions of the culture solution were plated onto a PAH-mineral agar medium, and the plates were incubated for 10 days at 30±1 ℃. Bacterial colonies producing clear zones were regarded as PAH-degrading bacteria. PAH-degrading endophytic bacterial colonies were purified on the same media by streaking three to four times in fresh media. Finally, the endophytic bacteria were stored on slants.

For the 16S rDNA analysis, genomic DNA was extracted, and 16S rDNA was amplified via polymerase chain reaction (PCR) with genomic DNA as a template and the universal bacterial primers, 27F (5'-GAGTTTGATCACTGGCTCAG-3') and 1492R (5'-TACGGCTACCTTGTTACGACTT-3'). The PCR mixture (25.0 μL) contained 0.5 μL of the template, 2.5 μL of 10× buffer (with Mg^{2+}), 1.0 μL of dNTP, 0.5 μL of F, 0.5 μL of R and 0.2 μL of polymerase. The PCR process was performed in a DNA engine thermal cycler (2720 Thermal Cycler, Applied Biosystems, USA) with a hot start at 94 ℃ for 4 min, followed by 30 successive cycles at 94 ℃ for 45 s, 55 ℃ for 45 s and 72 ℃ for 1 min; and then a final extension at 72 ℃ for 10 min. The amplification products were purified with a DNA purification kit (Sangon Biotech, China), and sequencing was performed at Sangon Biotech (Shanghai) Co., Ltd. The 16S rDNA sequence was compared against the GenBank database via the U.S. National Center for Biotechnology Information (NCBI) Blast program. The endophytic strain was identified as *M. extorquens* based on physiological characteristics and 16S rDNA sequence homology analysis results and named M. extorquens C1. This isolate was deposited at China Center for Type Culture Collection, under accession number CCTCC M 2019060.

B1: *Pseudomonas aeruginosa* J1

One gram of PAH-contaminated soil was mixed with 100 mL of sterile distilled water and agitated at a speed of 100 rpm in a shaker for 15 minute. After allowing the mixture to settle for 1 minute, 1 mL of the supernatant was plated onto a PAH-mineral agar medium, and the plates were incubated for 48 hours at 30±1 ℃. Bacterial colonies producing clear zones were regarded as PAH-degrading bacteria. PAH-degrading bacterial colonies were purified on the same media by streaking three to four times in fresh media.

The identification method for the strain utilizing 16S rDNA was conducted as described above. The isolate exhibited 99% similarity to *Pseudomonas aeruginosa* and has been designated as *Pseudomonas aeruginosa* J.

M2: Specific operating conditions for HPLC

Agilent 1260 HPLC (USA):

Column: 4.6×250 mm reverse-phase PAH column.

Column temperature: 40 ℃.

Mobile phase: distilled water and HPLC-grade methanol at a ratio of 20:80 (v:v).

Flow rate:1.00 mL/min.

Injection volume: 20 μL.

Detector: UV detector at 225 and 244 nm.

M3: PCR and Sequencing analysis

Amplicon Generation

16S rRNA/ITS genes of distinct regions (16SV4/16SV3-V4/16SV4-V5, ITS1/ITS2, Arc V4-V5) were amplified used specific primer (16SV3-V4:341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3')) with the barcode. All PCR reactions were carried out with Phusion High-Fidelity PCR Master Mix (New England Biolabs).

PCR Products quantification and qualification

Mix same volume of 1X loading buffer (contained SYB green) with PCR products and operate electrophoresis on 2% agarose gel for detection. Samples with bright main

strip between 400-450bp were chosen for further experiments.

PCR Products Mixing and Purification

PCR products were mixed in equidensity ratios. Then, mixture PCR products were purified with Qiagen Gel Extraction Kit (Qiagen, Germany).

Library preparation and sequencing

Sequencing libraries were generated using TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina MiSeq platform and 300 bp paired-end reads were generated.

Data analysis

Paired-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence. Paired-end reads were merged using FLASH[1], a very fast and accurate analysis tool, which was designed to merge paired-end reads when at least some of the reads overlapped the reads generated from the opposite end of the same DNA fragment, and the splicing sequences were called raw tags. Quality filtering of the raw tags was performed under specific filtering conditions to obtain high-quality clean tags according to the QIIME[2] quality control process. The tags were compared with the reference database (Gold database) using the UCHIME algorithm (UCHIME Algorithm) to detect chimera sequences, and then the chimera sequences were removed[3]. Then, the effective tags are finally obtained.

Sequence analysis was performed by UPARSE software (UPARSE V8.1.1861). Sequences with ⩾97% similarity were assigned to the same OTUs (operational taxonomic units). Representative sequences for each OTU were screened for further annotation. Taxonomic assignment was performed through the SILVA reference database by uclust at the 90% threshold.

Alpha diversity was applied to analyze the complexity of species diversity for a sample through 3 indices, including Chao1, Shannon, and Simpson. All these indices in our samples were calculated with QIIME (Version 1.9.1) and displayed with R software.

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