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Metagenomic Analysis and Core Flooding Reveals the Indigenous Bacterial Community Information and MEOR Potential of the Main Water-Drive Low-Permeability Reservoir in the Ordos Basin

Yang Wang,* Jinyu Yang, Xiaoxue Hu, Yi Wang, Senqiang Qin, Jingjing Fan, and Meipeng He

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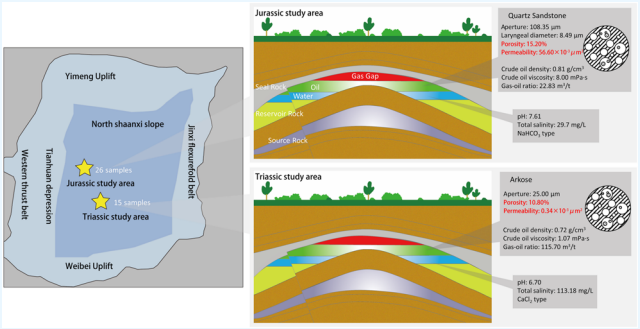
ABSTRACT: Oil recovery decreased with prolonged waterflood development in the main reservoirs in the Ordos Basin, and the extraction of the remaining oil was gradually difficult. To exploit oil-producing potential through microbial enhanced oil recovery (MEOR), bacterial communities of 26 samples from Yan 9, 10 layers, and 15 samples from Chang 6 layers were analyzed based on high-throughput sequencing. 1578 and 3581 amplicon sequence variants were obtained from Jurassic and Triassic samples, and α diversity analysis showed that dominant bacterial genera existed distinctly in both study areas. The distribution of the Jurassic bacteria community differed from that of the Triassic, revealed by a principal coordinate analysis. *Pseudomonas* (15.74%) and *Sulfurospirillum* (9.94%) were identified as the dominant bacteria in the Jurassic study areas, as well as *Pseudomonas* (33.54%) and *Acinetobacter* (11.41%) were the main bacteria in the Triassic reservoirs. Co-occurrence network analysis indicated that the Jurassic and Triassic study areas had both complex and unstable bacterial consortiums, which were closely connected with *Proteobacteria* and *Firmicutes*, respectively. The different development strategies and reservoir properties led to the discrepancy in indigenous bacteria distribution between the Jurassic and Triassic study areas. However, some bacteria that have been reported to have oil-displacing ability, such as *Pseudomonas*, *Halomonas*, *Acinetobacter*, *Marinobacterium*, and *Marinobacter*, were found in both regions, suggesting that these bacteria had extensive adaptability. Among them, the utilization of functional bacteria of *Proteobacteria* and *Firmicutes* might be conducive to enhancing oil recovery stably. Based on this, *Pseudomonas aeruginosa* PA2 was isolated from study areas and enhanced oil recovery by 17.85 and 11.89% during Jurassic and Triassic core flooding tests, respectively.



1. INTRODUCTION

Social development was highly dependent on the exploitation and utilization of petroleum resources, leading to the decrease of conventional petroleum reserves and the increasing attention to low-permeability oil reservoirs.[1−3] Jurassic Yan 9 and Yan 10 layers and the Triassic Chang 6 layer were the main oil-producing areas of the Ordos Basin, the biggest low-permeability oil resource development base in China with petroleum reserves of more than 68.3×10^8 tons.[4−6] After a long-term waterflood development, most of the reservoirs in the Jurassic Yan'an Formation and Triassic Yanchang Formation have entered the mid or high-water cut stage, and oil recovery has plummeted.[7,8] It was an urgent problem to improve oil recovery in water-drive low-permeability reservoirs.[9,10]

Microbial enhanced oil recovery (MEOR) was driven by microbial activities and their functional metabolites, especially indigenous bacteria with high environmental adaptability.[11,12] The mobility of petroleum and the relationship between oil−water and rock were improved by these indigenous oil-displacing bacteria, enhancing oil recovery.[13,14] Nowadays, the application potential of MEOR in low-permeability reservoirs has garnered increasing attention.[15,16] However, the lack of understanding of microbial community distribution, potential indigenous oil-displacing bacteria, and oilfield application potential hindered MEOR strategy optimization in water-driven low-permeability reservoirs of the Ordos Basin.

In this paper, the bacterial community distribution and interrelation were revealed based on high-throughput sequencing and annotation, an indigenous oil-displacing bacterium was directed to be screened, and the associated practical potential of oil recovery was evaluated by core flooding. This study provided a scientific basis for developing targeted MEOR strategies in water-flooded low-permeability reservoirs in the Ordos Basin.

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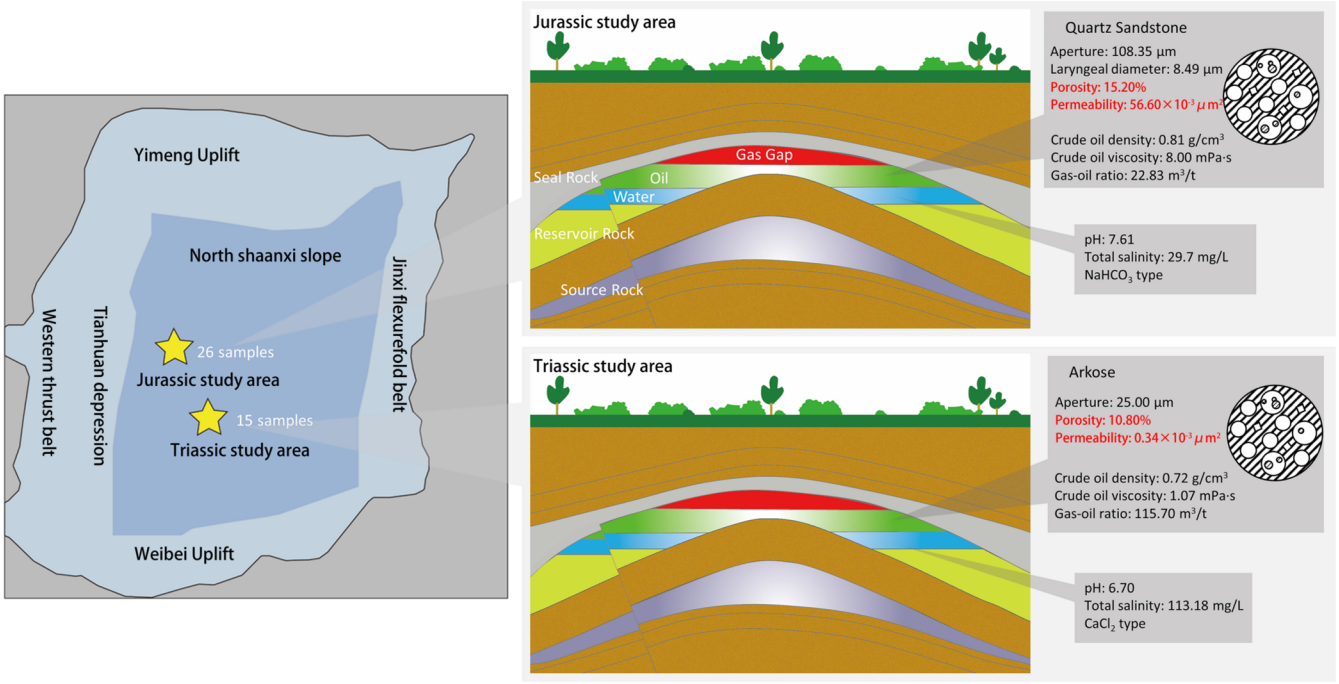

Figure 1. Reservoir characteristics of the investigation areas and physicochemical characteristics of samples.

2. MATERIALS AND METHODS

2.1. Sample Collection and Processing. Forty-one oil−water samples were collected from different sampling wells in the main oil-producing reservoirs of Jurassic Yan 9−10 layers and Triassic Chang 6 layer of the Ordos Basin. All samples were stored in sterile closed containers at 4 °C for further analysis. By mixing the oil and water phases and delaminating them again, the water phase in each sample was retained and transferred into a new sterile centrifuge tube. The bacterial cells were harvested through centrifugation at 4 °C and 12,000 rpm for 20 min.

2.2. DNA Extraction, Assembly, and High-Throughput Sequencing. DNA was extracted based on the SDS method, and the DNA concentration was adjusted to 1 ng/μL with sterile water after passing the agarose gel electrophoresis test. Using diluted genomic DNA as a template, the feature gene fragments were amplified using 16S V4 region-specific primers 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3′) with a barcode. After the PCR products were qualified by 2% agarose gel electrophoresis, the same amount of mixed samples were carried out according to the product concentration, and the mixed sample segments were recovered after the second test. NEBNext Ultra IIDNA Library Prep Kit was used to construct the library, and the constructed library was quantified by Qubit and Q-PCR. After the library was qualified, NovaSeq6000 was used for sequencing.

2.3. Data and Statistical Analysis. Each sample data was separated from the disembarkation data according to the barcode sequence and PCR-amplified primer sequence. After the barcode sequence and primer sequence were truncated, the sample reads were spliced using FLASH (V1.2.11) software. Subsequently, fastp software was used to conduct quality control, and Vsearch software was used to remove the chimeras to obtain the Effective Tags. Amplicon sequence variants (ASVs) were obtained through denoising by the DADA2 module in QIIME2 software, controlling consistency as 100%. QIIME2 software was also used for ASVs annotation and calculation of observed ASVs, Shannon, Simpson, Chao 1 index, and goods coverage for α-diversity analysis. Principal coordinate analysis (PCoA) was conducted based on Bray−Curtis. PICRUSt2 was used for functional annotation of functional genes of bacterial communities, based on the KO database. Correlation networks within the two bacterial communities were constructed based on the Spearman algorithm. R software was used to visualize the analysis results.

The Tukey and Kruskal−Wallis tests were used to evaluate the α-diversity index of each group. Statistical tests were conducted for PCoA using adonis, and the confidence level of the confidence ellipse was 0.95.

2.4. Bacterial Isolation, Identification, and Oil-Displacement Capability Characterization. Five milliliters of Jurassic and Triassic oil−water samples (5 mL) were added in 90 mL of the mineral salt medium as an inoculant in sterile conical flasks, and the cultivation was conducted at 37 °C and 180 rpm for 3 days. After serial dilution, bacteria were isolated and purified in Luria−Bertani plates. 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGCTACCTTGTTACGACTT-3′) primers were employed to amplify the bacterial 16S rRNA gene sequence to compare with other sequences in GenBank and construct the phylogenetic tree in MEGA 7 software with the neighbor-joining method.[17] According to Albasri, the bacterial oil displacement and emulsification capability were evaluated based on the oil-spreading and emulsification index (E_{24}) measure.[18] Mineral salt medium (1 L): 1 g of $(NH_4)_2SO_4$, 7 g of K_2HPO_4, 3 g of KH_2PO_4, 2.25 g of $NaNO_3$, and 10 g of NaCl (pH 7.0−7.2). Luria−Bertani medium (1 L): 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl (pH 7.0). All of the media were autoclaved at 121 °C for 30 min.

2.5. Core Flooding Test. Two cores with a permeability of 33.20×10^{-3} and 5.12×10^{-3} μm^2, respectively, were used to simulate the reservoir rock environments of the Jurassic and

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Triassic. Prior to experiments, the core samples were oven-dried to constant weight and their dry weights were measured, followed by vacuum saturation with formation water to determine pore volume. Two cores were mounted in a core holder and subjected to reservoir conditions of 57 °C and 3 MPa and 70 °C and 12 MPa to simulate Jurassic and Triassic environments, respectively. After waterflooding to 98% water cut, 1 PV of indigenous bacterial culture (PA2 strain, 1% concentration) was injected, followed by secondary waterflooding until no additional oil was produced, with oil recovery monitored throughout the experiment.

3. RESULTS AND DISCUSSION

3.1. Geological Profile of Sample Sites and Physicochemical Properties of Oil−Water Samples. The Jurassic study area was located in the middle and north of the North Shaanxi Slope of the Ordos Basin, Yulin, Shaanxi, China, and the Triassic study area was located in the west of the North Shaanxi Slope, Yan'an, Shaanxi, China. Characteristics of reservoirs and samples in the study areas are shown in Figure 1 (the sampling coordinates involved confidentiality). The Jurassic Yan 9−10 layers were mainly composed of quartz sandstone with an average porosity of 15.20% and permeability of 56.6×10^{-3} μm^2. For the Triassic Chang 6 study areas, the main reservoir rock types were fine, densified siltstone and shale, with average porosity and permeability of 10.80% and 0.34×10^{-3} μm^2, respectively. The reservoir temperature was about 57.2 °C in the Jurassic study area and 69.7 °C in the Triassic study area. The two study areas have different formation water types ($NaHCO_3$ in the Jurassic and $CaCl_2$ in the Triassic) but similar pH (6.7−7.61) and low salinity content (29.7−113.18 g L^{-1}).

Both the Jurassic and Triassic study areas belong to low-permeability reservoirs in terms of oil production cognition, and the physical properties in the Jurassic study areas were slightly better than the Triassic.[5,6] The moderate pH allowed most bacteria to survive, which was conducive to the protection of bacterial diversity in the Jurassic and Triassic reservoirs. Compared with the Jurassic reservoir, the Triassic reservoir presented more obvious characteristics of high temperature and high salinity, which were probably suitable for the survival of thermophilic, halophilic, or high-stress resistant strains.

3.2. α-Diversity and β-Diversity Characteristics in Jurassic and Triassic Study Areas. A total of 1,764,938 effective reads were identified from 26 Jurassic samples, and a total of 1,101,627 effective reads were found from 15 Triassic samples, which were annotated as 1,578 ASVs and 3,581 ASVs, respectively. The rarefaction curves showed that, with the increase in sample size, ASV amount, and α-diversity index gradually stabilized, indicating that the sample size was sufficient to reflect the distribution of bacterial communities in the study areas (Figure S1).[19] Average ASV number and Shannon, Simpson, and Chao 1 indices were used to evaluate community richness and diversity from Jurassic and Triassic reservoirs (Tables 1 and S1). The similar Shannon and Simpson index between the two study areas indicated that Jurassic and Triassic reservoirs have a similar bacterial richness, and both of them have dominant species. The bigger average ASV number and Chao 1 index in Triassic samples, compared with the Jurassic, showed that bacteria with low abundance gathered in Triassic reservoirs. The differences in the bacterial structure between samples were estimated by PCoA dimensionality reduction analysis (Figure 2). PC1 explained 35.38% of the variation, and PC2 explained 10.65% of the variation. Samples from Jurassic reservoirs were primarily gathered in the third quadrant, and Triassic samples were mainly concentrated in the first quadrant, displaying an obvious discrepancy in indigenous bacterial distribution between the two groups of samples ($r^2 = 0.210$, $P = 0.001$).

Table 1. Amplicon Sequence Variants (ASVs) and Average Value of α Diversity Indices in Jurassic and Triassic Samples

sample	average ASVs	Shannon	Simpson	Chao 1	goods coverage
jurassic samples	321.19	4.56	0.83	329.69	1.00
triassic samples	557.80	4.17	0.76	560.31	1.00

3.3. Distribution Characteristics of Bacterial Communities in Jurassic and Triassic Study Areas. Based on taxonomic composition and abundance analysis, 465 genera and 723 genera were detected in Jurassic and Triassic bacterial samples, respectively, and 225 genera were found in both bacterial communities (Figure 3a). The relative abundance of the top 30 genera shared by Triassic and Jurassic reservoirs is exhibited in Figure 3b. It was *Pseudomonas*, the most abundant genera in the two study areas, accounting for 15.74 and 33.54% in the Jurassic and Triassic samples, respectively. *Sulfurospirillum* (9.94%), *Desulfotignum* (8.66%), and *Halomonas* (6.80%) were also prominently distributed in the Jurassic research areas, and *Acinetobacter* (11.41%), *Marinobacterium* (7.58%), and *Marinobacter* (7.51%) were also abundant in Triassic study areas. The top 10 genera that were only found in Jurassic reservoirs and only found in Triassic reservoirs accounted for 2.81 and 1.78% in the two areas, respectively, such as *Desulfonatronum* in Jurassic samples and *Epulopiscium* in Triassic samples, which reflected the fluid characteristics of the reservoir to a certain extent (Figure 3c).

Although there were obvious differences in the distribution proportion of bacterial genera between the Jurassic and Triassic samples, the bacteria found in both two study areas accounted for 76.70 and 88.64% of Jurassic and Triassic indigenous bacteria, respectively, such as *Pseudomonas*, *Halomonas*, *Acinetobacter*, *Marinobacterium*, and *Marinobacter*, which have a general adaptation in Jurassic and Triassic reservoirs and have reported as oil-displacing bacteria (Figure S1).[19−24] The cell size range of these genera mentioned above was usually from 0.1−1.6 to 1.0−5.0 μm; thus, bacteria could successfully pass through the reservoirs with an average pore throat of 25 μm.[24−26] A wide range of temperature and pH tolerance (15−40 °C, pH 4−10) allowed the bacteria to adapt to a variety of multifarious fluid environments, which could serve as bacterial resources for MEOR in Jurassic and Triassic oilfields.[25,26] In addition, many nitrate and sulfate metabolizing bacteria were only detected in Jurassic samples, and acidophilic and acid-forming bacteria gathered in the Triassic, suggesting the potential enrichment of nitrate, sulfate, and acidic material.[27,28]

3.4. Functional Enrichment Analysis of Bacterial Community. The taxonomic structure and community function of the bacterial communities were correlated, and the analysis of major community functions in the Jurassic and Triassic reservoir study areas was based on the annotation of functional genes in the KO database and the mapping of functional annotation cluster heat maps (Figure 4). In the

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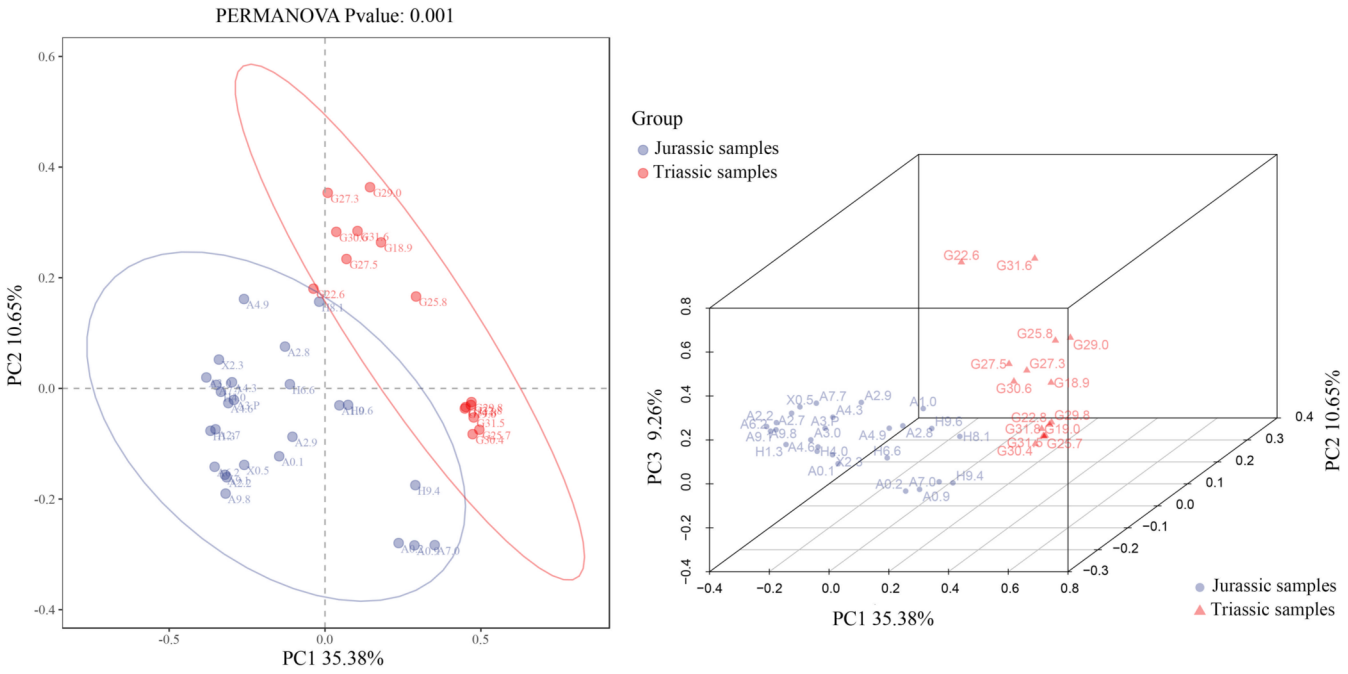

Figure 2. PCoA analysis based on bacterial communities showed the differences and similarities between Jurassic and Triassic samples.

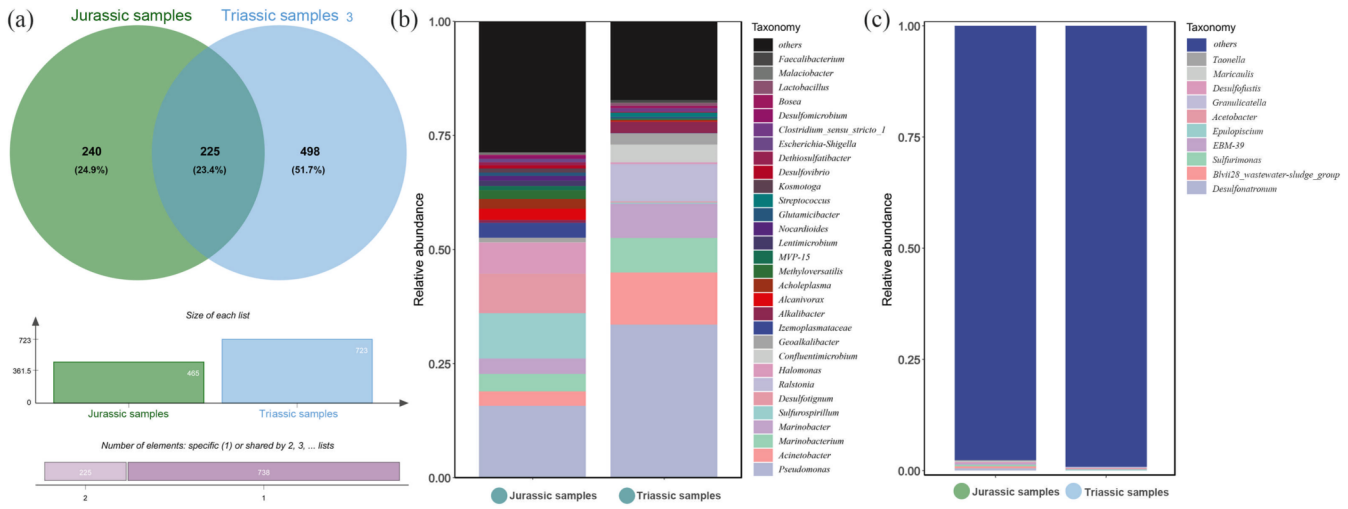

Figure 3. (a) Venn distribution of ASVs in Jurassic and Triassic study areas; (b) the distribution of coexisting bacteria genera (top 30) in the two study areas; and (c) the distribution of unique bacteria genera (top 10) in the two study areas.

same study area, there was no obvious split of community function distribution between different sampling Wells. The main bacterial community functions in the Jurassic reservoir were concentrated on amino acid metabolism, carbohydrate transport, fatty acid biosynthesis, and iron complex transport. The main bacterial community functions in the Triassic reservoir were mainly amino acid metabolism, carbohydrate transport, fatty acid biosynthesis, peptide/nickel transport, bacterial chemotaxis, and construction of a two-component signal transduction system.

The highly enriched functions of amino acid metabolism (K01995−K0999, K02030, K02029, K01915), carbohydrate transport (K02057), and fatty acid biosynthesis (K00626, K00059) in the two study areas not only have an important impact on the survival and development of bacteria but also have an important impact on the survival and development of bacteria.[29,30] It also affects the production of biosurfactants, such as glycolipids and lipopeptides.[31,32] To a certain extent, the functional differences in microflora between the two reservoirs reflect the differences between the two types of reservoir environments.[33] Heavy metal metabolism (iron complex transport K02013\K02015\K02016, iron complex outer membrane receptor protein K02014, ferredoxin oxidoreductase K03738, etc.) and methane metabolism (K03388 heterodisulfide reductase subunit A2) were highly enriched in the Jurassic study area. It indicates that there may be an accumulation of heavy metals and methane in the Jurassic area. Fatty acyl-CoA synthesis (K00666), bacterial chemotaxis (K03406\K02557), and two-component systems (K00626, K02483, K01915, K03406, and K07165) were highly enriched in the Triassic reservoir research area. It was suggested that the fluid environment in the Triassic research area was complicated, and the liquid environment was acidic as a whole.

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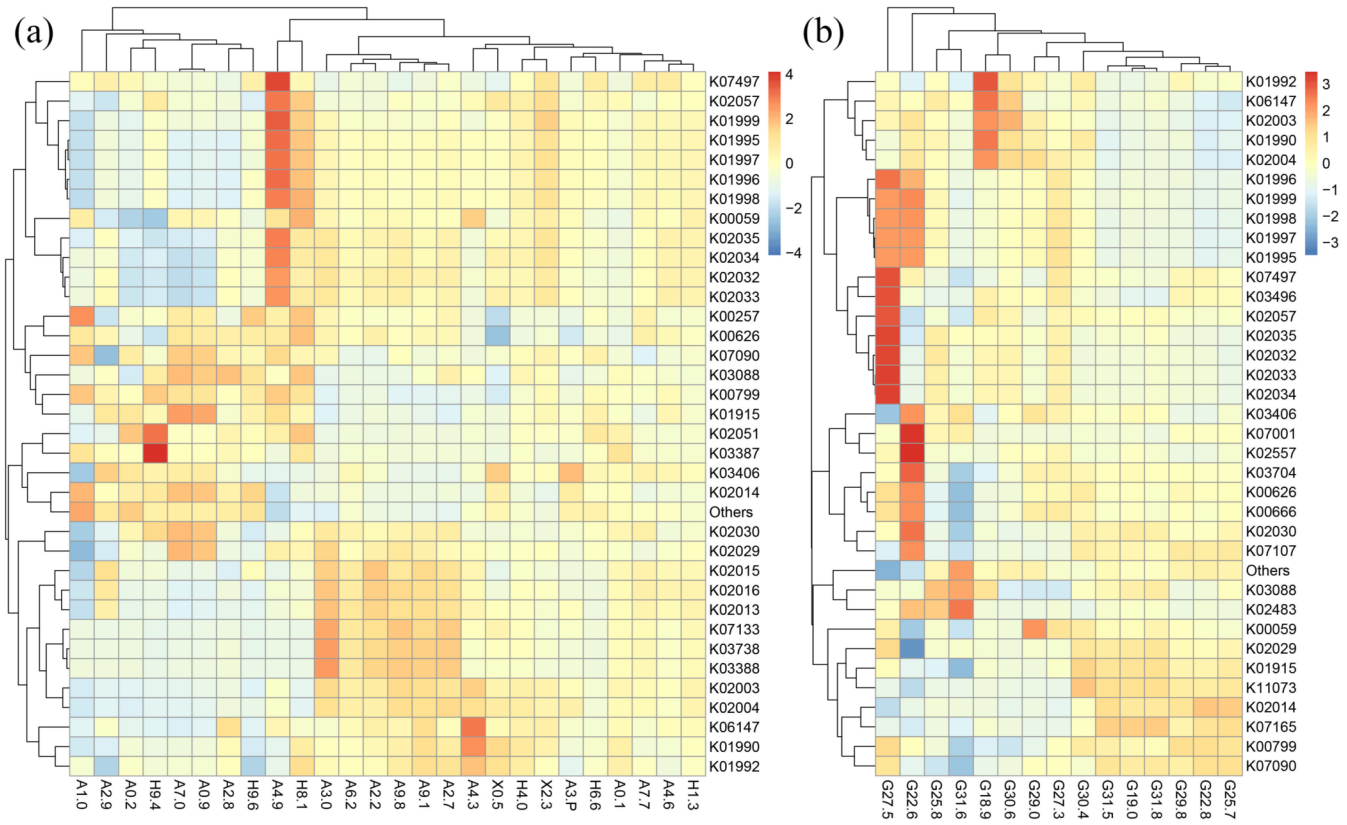

Figure 4. Relative abundance clustering heat map of bacterial functional annotation (Top 35) in the (a) Jurassic and (b) Triassic study areas.

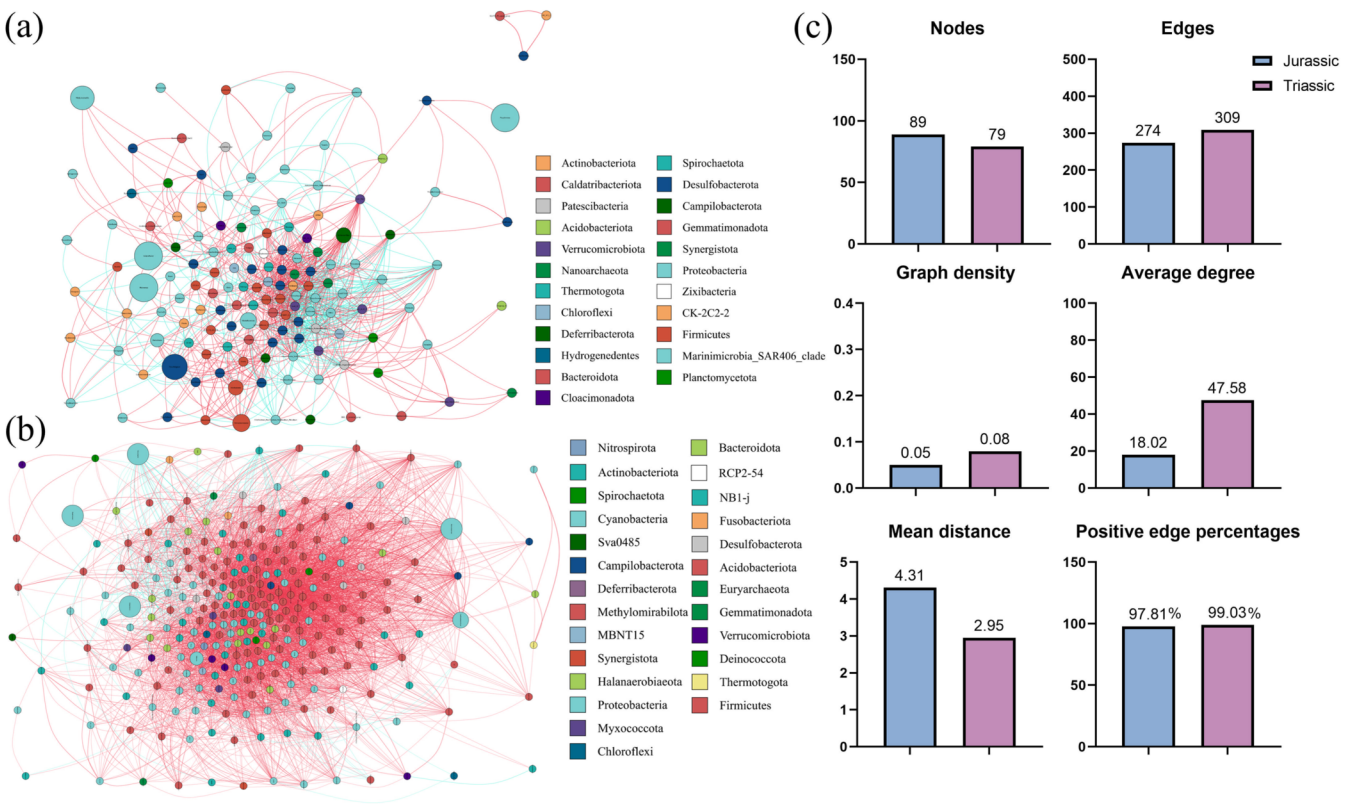

Figure 5. Bacterial community correlation network in (a) Jurassic and (b) Triassic study areas and (c) topological properties.

3.5. Co-occurrence Network Analysis. Co-occurrence network analysis based on Spearman correlation index calculations is shown in Figure 5. Compared with the Triassic, the Jurassic network has more nodes but exhibited fewer edges

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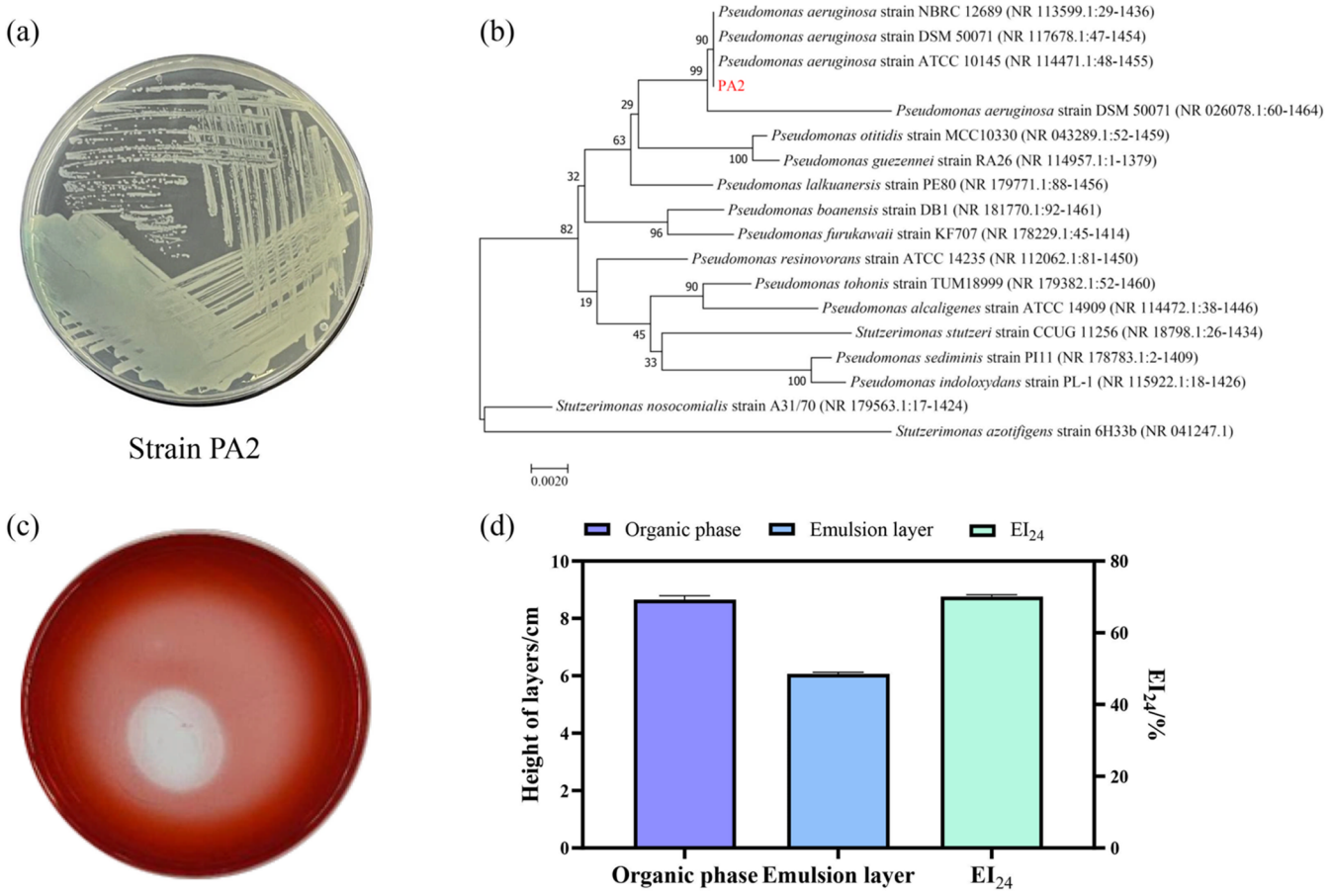

Figure 6. (a) The typical colony; (b) phylogenetic tree stem from the 16S rRNA gene sequence; (c) oil-spreading result; and (d) emulsification index of the strain PA2.

(decreased by 11.33%), network density (decreased by 37.5%), average connectivity (decreased by 63.13%), and larger mean distance (decreased by 46.10%). In addition, the proportion of positive edges in the Jurassic and Triassic bacterial networks was 97.81 and 99.03%, respectively. *Maricaulis*, *Polycyclovorans*, *Pseudohongiella*, *Thalassobaculum*, *Pusillimonas*, *Nitratireductor*, *Desulfomicrobium*, and *Thermovirga* were the main genera in the Jurassic bacterial network, and 78.47% of the connection was related to *Proteobacteria*. The main genera in the Triassic bacterial network were *Blautia*, *Dialister*, *Dorea*, *Eubacterium_hallii_group*, *Roseburia*, *Enhydrobacter*, *Fusicatenibacter*, *Subdoligranulum*, *Thalassospira*, and *Anaerostipes*, and 82.20% of the connections were connected with *Firmicutes*.

In the oil reservoir ecosystem, bacteria were connected through the exchange of matter, energy, and information, forming complex interactions, including predation, competition, mutualism, disinterested symbiosis, etc.[34,35] This complex ecological relationship can be expressed as a network, with species as nodes and relationships between species as ties. This was the basis for characterizing species interactions and ecosystem dynamics. The complexity and stability of ecological networks were the key to the macro evaluation of ecological networks.[36,37] Although the bacterial communities in the Triassic showed high complexity, both the Jurassic and the Triassic had high proportions, indicating that cooperative interactions between bacteria indicated cooperative interactions between bacteria, indicating low community stability.[38,39] The Jurassic network was mainly related to *Proteobacteria* and the Triassic network was related to *Firmicutes*, which indicates that MEOR can focus on these bacteria and help to improve the stability of the community where the oil-flooding bacteria reside.

3.6. MEOR Potential Investigation by Indigenous *Pseudomonas aeruginosa*. Several bacteria were isolated from oil−water samples in study areas; as mentioned above, *Pseudomonas* was an indigenous genus that was highly adaptable in Ordos Jurassic and Triassic water-driven low-permeability reservoirs with oil-displacing potential. Thus, strain PA2 was directed to be screened. The single colony of strain PA2 was greenish-yellow, smooth, and roundish with a regular margin (Figure 6a). Clustering analysis of 16S rDNA gene sequences suggested that there was extensive homology between strain PA2 and *P. aeruginosa* (Figure 6b). The oil-displacing capability assessment demonstrated that the fermentation broth of strain PA2 exhibited significant interfacial activity, displaying a positive oil-spreading effect and achieving an E_{24} value of 70.11% (Figure 6c,d). Furthermore, the results of the core flooding test showed that the oil recovery enhanced by strain PA2 in simulated Jurassic and Triassic reservoir environments was 17.85 and 11.89%, respectively (Figure 7).

The efficiency of MEOR was fundamentally determined by both the oil-displacing capacity of the bacterial strains and their compatibility with reservoir geology, fluids, and biological environments, which explained the preferential use of indigenous microorganisms.[11,13] *Pseudomonas* was detected at

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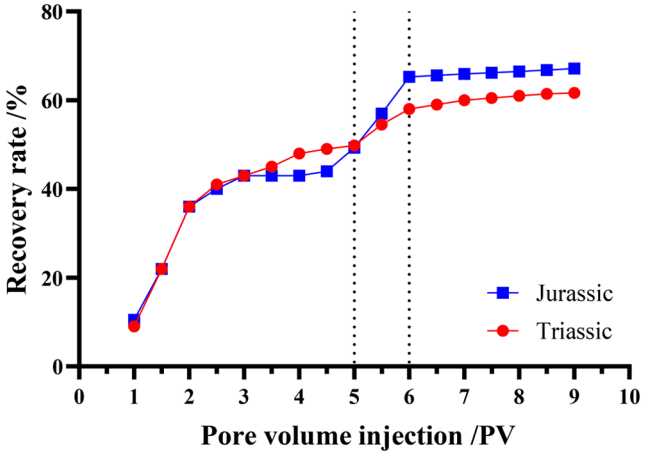

Figure 7. Recovery rates during core flooding tests in simulated Jurassic and Triassic environments.

abundances of 15.74 and 33.54% in Jurassic and Triassic study areas, indicating its strong competitiveness among the native bacteria and high adaptability in practical reservoir environments. As a member of the prevalent indigenous *Pseudomonas*, strain PA2 has been demonstrated to possess superior oil-displacement and petroleum-emulsifying capabilities compared to previously reported *Parapedobacter indicus* and *Paenibacillus antarcticus*.[40,41] In simulated Jurassic and Triassic core flooding, oil recovery enhancement by strain PA2 both exceeded 10%, implying the technical effectiveness and environmental applicability of the MEOR in the two study areas. The Jurassic system showed higher oil recovery enhancement than the Triassic system, which is attributed to better reservoir properties and closer microbial interactions.

4. CONCLUSION

Bacterial consortia in Jurassic and Triassic study areas have similar community richness and different community structures. *Pseudomonas*, *Halomonas*, *Acinetobacter*, *Marinobacterium*, and *Marinobacter*, which have been confirmed with oil-displacing ability, were abundant in both study areas, indicating that those bacteria had wide adaptability in the Ordos basin and could be used in the MEOR of low-permeability reservoirs. *Proteobacteria* and *Firmicutes* were confirmed as the primary communicators in Jurassic and Triassic bacterial communities, respectively, via co-occurrence network analysis; thus, promoting those bacteria could be beneficial to community stabilization and continuous enhancement of oil development. The isolation and core flooding validation of *P. aeruginosa* PA2, which enhanced oil recovery by 17.85% in the Jurassic and 11.89% in the Triassic, indicated the potential for the effective implementation of MEOR. These findings provide critical insights into the microbial ecology of water-flooded tight reservoirs and support the development of targeted MEOR strategies by leveraging indigenous functional bacteria.

■ ASSOCIATED CONTENT

Data Availability Statement

The data sets generated and/or analyzed during the current study are available in the ZENODO repository, 10.5281/zenodo.14607872.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.5c01671.

Amplicon sequence variants (ASVs) and α-diversity indices of the sampled well areas (Table S1); column chart of relative abundance of species at genus level in Jurassic samples and Triassic samples (top 30) (Figure S1). (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Yang Wang − *No. 6 Oil Production Plant, PetroChina Changqing Oilfield Company, Yulin 718500 Shaanxi, China;* orcid.org/0009-0005-4158-2515; Phone: 86-29-18691839723; Email: 1296877471@qq.com

Authors

Jinyu Yang − *No. 6 Oil Production Plant, PetroChina Changqing Oilfield Company, Yulin 718500 Shaanxi, China*

Xiaoxue Hu − *No. 6 Oil Production Plant, PetroChina Changqing Oilfield Company, Yulin 718500 Shaanxi, China*

Yi Wang − *No. 6 Oil Production Plant, PetroChina Changqing Oilfield Company, Yulin 718500 Shaanxi, China*

Senqiang Qin − *No. 6 Oil Production Plant, PetroChina Changqing Oilfield Company, Yulin 718500 Shaanxi, China*

Jingjing Fan − *No. 6 Oil Production Plant, PetroChina Changqing Oilfield Company, Yulin 718500 Shaanxi, China*

Meipeng He − *No. 6 Oil Production Plant, PetroChina Changqing Oilfield Company, Yulin 718500 Shaanxi, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.5c01671

Author Contributions

Yang Wang, J.Y., and X.H. conceptualized the project, sourced project funds, and designed the methodology. Yi Wang collected and analyzed data. S.Q. supervised data collection and analysis. J.F. and M.H. interpreted the results and wrote the manuscript. All authors read and approved the final manuscript.

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Notes

The authors declare no competing financial interest.

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