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Bioaugmentation enhance the bioremediation of marine crude oil pollution: Microbial communities and metabolic pathways

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ABSTRACT

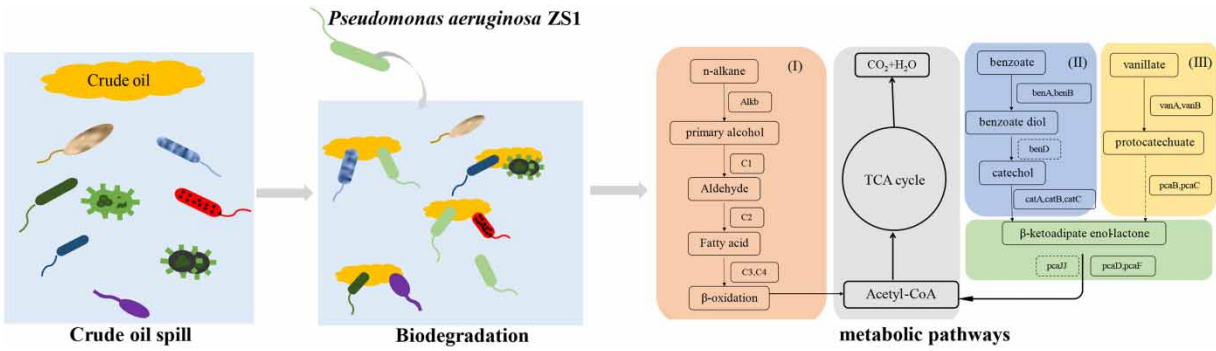
Bioaugmentation is an effective strategy used to speed up the bioremediation of marine oil spills. In the present study, a highly efficient petroleum degrading bacterium (*Pseudomonas aeruginosa* ZS1) was applied to the bioremediation of simulated crude oil pollution in different sampling sites in the South China Sea. The metabolic pathways of ZS1 to degrade crude oil, the temporal dynamics of the microbial community response to crude oil contamination, and the biofortification process were investigated. The results showed that the abundance and diversity of the microbial community decreased sharply after the occurrence of crude oil contamination. The best degradation rate of crude oil, which was achieved in the samples from the sampling site N3 after the addition of ZS1 bacteria, was 50.94% at 50 days. C13 alkanes were totally oxidized by ZS1 in the 50 days. The degradation rate of solid n-alkanes (C18–C20) was about 70%. Based on the whole genome sequencing and the metabolites analysis of ZS1, we found that ZS1 degraded n-alkanes through the terminal oxidation pathway and aromatic compounds through the catechol pathway. This study provides data support for further research on biodegradation pathways of crude oil and contributes to the subsequent development of more reasonable bioremediation strategies.

Key words: bioremediation, crude oil degradation, microbial community structure, *Pseudomonas aeruginosa* ZS1, whole genome sequencing

HIGHLIGHTS

- *Pseudomonas aeruginosa* ZS1 is an effective strain for bioremediation of crude oil.
- ZS1 has an advantageous position in the bioremediation process.
- Degradation pathway of n-alkanes and aromatic compound by ZS1 was revealed.

GRAPHICAL ABSTRACT



1. INTRODUCTION

As a traditional energy fuel and industrial raw material, petroleum is still the most dominant energy source in the world nowadays. Since crude oil is transported over long distances mainly by ships and other vessels at sea, the frequency of crude oil spills has increased. Over the past decade, more than 1 billion gallons of oil have been spilled worldwide, and 6 million tons of oil enter the ocean each year (Zhang *et al.* 2019). Crude oil includes a complex mixture of various hydrocarbon compounds, mainly in the form of aliphatic and aromatic compounds (Al-Sayegh 2016). Compared to diesel fuel, crude oil has a significantly lower content of n-alkanes and a significant increase in aromatic and heterocyclic content, which increases the difficulties for bioremediation (Hong *et al.* 2017). Crude oil is also denser and more viscous than diesel fuel and can be deposited on shorelines and shoreline resources in thick viscous layers, which may disrupt or completely prevent normal biological exchange processes with the environment (Brito *et al.* 2006; Fakhrzadegan *et al.* 2019). The dispersion, emulsification, and evaporation of crude oil after the spill also can cause irreparable damage to the marine ecosystem (Jenkins & Adams 2011).

The main treatment methods currently available for oil spill contamination are physical, chemical, and biological methods (Ghoreishi *et al.* 2017; Kachieng'a & Momba 2017). Physical and chemical techniques are generally suitable for treating high concentrations of oil spills, such as floating and dispersed oil, in the early stages of pollution, but are less effective in removing emulsified and dissolved oil. Biological methods are generally used for post-remediation of oil spills and have the advantages of low cost, low environmental stress, and complete restoration of contaminated sites (Lovley 2003; Hamdan *et al.* 2019; Chen *et al.* 2020). In the biological method, microbial degradation is the main mechanism for removing petroleum hydrocarbons from contaminated environments. Among the various microorganisms that utilize petroleum hydrocarbons, bacteria are considered to be the most active and important ones (Das & Chandran 2011). As a bioremediation method, bioaugmentation technology has two main approaches: the addition of dominant strains and microbial co-metabolism (Shi *et al.* 2020). One of the advantages of bioremediation is that the degradation process can start immediately when a specific microbial degrader is introduced (Wu *et al.* 2019). Shi *et al.* (2020) found that 71.86% degradation of diesel fuel by *Halomonas* sp. P1 was achieved in 45 days biofortification, which was higher than 38% without biofortification. Rodrigues *et al.* (2020) found a significant increase in hydrocarbon degradation by biofortification by studying the distribution of cells during the growth of bacteria in mineral media containing cetane.

The South China Sea is relatively rich in oil and gas resources, which are being exploited by China and neighboring countries. The total annual exploitation can reach more than 50 million tons, with more than 1000 offshore drilling rigs. The large-scale oil and gas exploitation and the construction of submarine oil and gas pipelines have promoted the economic development of the world and China, but there is also a high risk of leakages, such as the Penglai 19-3 oil spill accident and the Gulf of Mexico Oil Spill (Wang *et al.* 2018; Hackbusch *et al.* 2020). It is crucial to understand the composition of the marine microbial community in the South China Sea and its response to marine oil spills for developing an effective and feasible oil spill remediation plan. In a previous study, we obtained an efficient oil-degrading bacterium *Pseudomonas aeruginosa* ZS1 that can produce surfactant (Liu *et al.* 2022). The surfactant produced by ZS1 can emulsify the oil droplets into smaller droplets, facilitating direct contact between the microorganisms and the oil droplets (Li *et al.* 2021; Liu *et al.* 2022).

Therefore, the objectives of this paper are: (i) to investigate the effects of oil spill pollution on deep-sea indigenous microorganisms in the South China Sea and their response to oil spill pollution; and (ii) to investigate the role of ZS1 in the remediation of marine oil spill pollution and metabolic pathways of crude oil.

2. MATERIAL AND METHODS

2.1. Sample collection and preparation

In total, five seawater samples were collected in a box corer during R/V Xiang Yang Hong 18 in July 2020 from the South China Sea (Shi *et al.* 2022). Five locations were investigated, and the locations of the sampling points are shown in Figure A1. The sampling depths of the samples are shown in Table A1. The samples (SS) included sediment and water, of which sediment accounted for 27 $\pm$ 3%. The collected samples were kept in the sampling tubes and placed in storage at 4 °C.

2.2. Mediums and oil biodegrading strains

The chemicals used in the experiments were at least of analytical purity grade. The crude oil was obtained from Venezuela (10°30′00 'N, 66°56′00 'W) with a density of 0.809075 g/mL. LB medium consisted of 10 g/L peptone, 5 g/L yeast powder and 5 g/L NaCl. 1 M of sodium hydroxide (NaOH) and hydrochloric acid (HCl) were added to the medium to keep the pH at 7.4.

The petroleum degrading bacteria *Pseudomonas aeruginosa* ZS1 used in the experiment was screened from seawater, which was taken from the oil pipeline explosion contaminated sea area in Qingdao, Shandong Province, China (36°4′12 'N, 120°22′48 'E) (Liu *et al.* 2022). The strain ZS1 was identified as *Pseudomonas aeruginosa*, belonging to the genus Pseudomonas, with 99% homology to *Peseudomona aeruginosa strain SH15 (MN999950.1)* (Liu *et al.* 2022).

2.3. Biodegradation of petroleum hydrocarbons

The experiments were divided into three groups. Both crude oil and ZS1 bacteriological solution were added to SS at a rate of 1%. Group 0 added only 50 mL SS as a blank control; group 1 added 50 mL SS and 5 mL of crude oil to simulate the marine environment contaminated by oil; group 2 added 50 mL SS, 5 mL of crude oil and 5 mL of ZS1 bacterial suspension to simulate the bioremediation of the oil spill. The experimental samples of each group at different sampling sites were named and are shown in Table 1. The experiments were all conducted in 100 mL Erlenmeyer flasks at 140 r/min and 16–18 °C for 50 d. Three parallel samples were prepared for all experiments.

2.4. Analytical instrumentation

2.4.1. Crude oil degradation rate determination

The samples from each group were incubated for 50 days before the determination of crude oil degradation rate. Firstly, the pH of the bacterial suspension was adjusted to below 2.0 with hydrochloric acid (v:v = 1:1). Then the residual oil in the sample was extracted with tetrachloroethylene and diluted 1000 times. Lastly, the crude oil content was determined by an Infrared oil meter (OIL-460, China). The degradation rate of crude oil was calculated according to Equation (1):

$$\text{Degradation} = \frac{C_0 - C_1}{C_0} \times 100\% \tag{1}$$

where C_0 is the initial crude oil concentration and C_1 is the residual oil concentration after degradation.

2.4.2. Community structure analysis

The bacterial suspension of different groups was filtered through 0.22 μm water system filter membrane. Each sample was stored in 5 mL cryopreservation tubes at −80 °C and sent to Shanghai Majorbio Bio-pharm Technology Co., Ltd for DNA extraction and high-throughput sequencing. After completing the genomic DNA extraction, the extracted genomic DNA was detected by 1% agarose gel electrophoresis. PCR was performed using TransGen AP221-02 (TransStart Fastpfu DNA Polymerase). All samples were subjected to the following experimental conditions, and three biological replicates were performed for each sample. PCR products from the same sample were mixed and detected by 2% agarose gel electrophoresis, and PCR products were recovered by gel cutting using the AxyPrepDNA Gel Recovery Kit (AXYGEN). Elution was performed with Tris-HCl. The PCR products were detected and quantified by QuantiFluor™ -ST Blue Fluorescence Quantification System (Promega). The structure and diversity of microbial communities were analyzed using the free online platform of Majorbio I-Sanger cloud platform.

Table 1 | Detailed table of sample grouping

Name of the sampling point	Group 0	Group 1	Group 2
QO2	C10	C11	C12
N3	C20	C21	C22
SO1	C30	C31	C32
SO3	C40	C41	C42
SO2	C50	C51	C52

Samples are named by Cmn, where m refers to different samples and n represents different treatments of the same sample.

2.4.3. Change in crude oil components

One microliter of the sample extracted with tetrachloroethylene was injected into a Shimadzu QP2010 SE gas chromatograph equipped with a capillary gas chromatographic column HP-5MS (0.25 μm × 30.0 m × 0.25 mm) and a Shimadzu mass selective detector, which was set to scan from 25 to 900 M/Z at a scan rate of 1.2 times per second. The injector inlet temperature was 280 °C and the detector interface temperature was 250 °C. The carrier gas was helium with a flow rate of 1 mL/min and a splitting ratio of 10:1.

2.4.4. Whole genome sequencing of *Pseudomonas aeruginosa* ZS1

Firstly, the strain ZS1 which was activated in LB medium was centrifuged for 20 min at 20,000 rpm by high-speed frozen centrifuge (D3024R, China) to harvest the precipitates. The high-throughput sequencing was conducted with Illumina Hiseq × 10 platform by Shanghai Majorbio Bio-pharm Technology Co., Ltd.

2.5. Statistical analysis

All experiments in this study were repeated at least three times. Statistical tests such as mean (±) standard deviation (SD) were calculated for each replicate using MS-Excel (2019). Graphs and tables were produced using MS-Excel (2010) and origin software (origin 2018 64bit). Graphs related to changes in community structure were performed using the online platform of Majorbio Cloud Platform (www.majorbio.com).

3. RESULTS AND DISCUSSION

3.1. Degradation performance analysis

After 50 days of biodegradation, the variation of crude oil in water is shown in Figure 1. It was found that C12 and C22 had higher degradation rates of 60.55 and 50.94%, respectively, followed by C52, C32, C42, and C11 samples with about 30 ~ 40%, and C51 had the lowest degradation rate of 3.20%. Compared with Group 1, the residual crude oil concentration of samples in Group 2 (incubating with ZS1 supplementation) was lower, indicating that the added petroleum-degrading bacterium ZS1 significantly improved the crude oil degradation capacity.

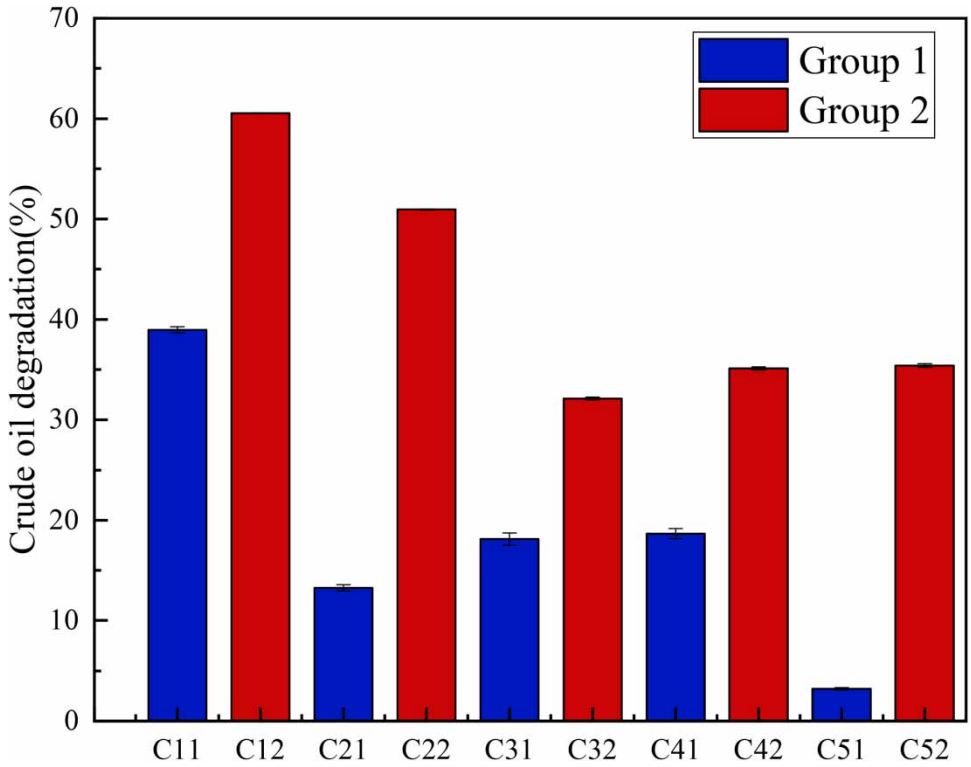


Figure 1 | Degradation rate of crude oil at 50 d at different sampling points.

3.2. Response of microbial communities to crude oil pollution

RDP classifier Bayesian algorithm was used to analyze the taxonomy of OTU representative sequences at 97% similarity level in Qiime platform. The rarefaction curve (Shannon index) indicates that the amount of sequencing data for each sample is large enough to reflect the vast majority of microbial diversity information. The change trend of community richness and diversity did not show much difference in different sampling sites. Species richness and evenness at the five sampling sites showed a significant decrease after the contamination of crude oil (Table A2).

Figure 2 illustrates the community composition of each sample at the genus level. In the original uncontaminated samples, the dominant microorganisms varied at each sampling site, strains *Actinomarinales* and *Sulfitbacter* were present at high levels as common dominant strains. In particular, the community diversity of C50 samples was significantly lower than that of the other four groups (C10, C20, C30, C40). In the samples contaminated by crude oil, the community abundance and diversity decreased significantly in all sampling sites. Among them, the abundance of *Marinobacter* increased significantly and became the new main dominant strain. In addition, the abundance of strains *Alcanivorax*, *Idiomarina*, and *Erythrobacter* varied among the sampling sites, but increased compared to the original seawater samples at each site. *Idiomarina* is a deep-sea bacterial strain associated with oil degradation (Wang *et al.* 2010; Malavenda *et al.* 2015; Fakhrzadegan *et al.* 2019), and the increase in the relative abundance of *Idiomarina* strains at each sampling site was due to a stress response by indigenous microorganisms after the oil spill contamination. After the addition of strain ZS1, the bacterial abundance decreased and the dominant colonies changed at all sampling sites. *Marinobacter*, *Alcanivorax*, and *Idiomarina* were still present as the dominant species. *Alcanivorax* and *Idiomarina* have been shown to be associated with alkane degradation (Rodrigue *et al.* 2020), while *Marinobacter* is associated with the degradation of aromatic hydrocarbons (Murphy *et al.* 2021). Furthermore, it was observed that the abundance of *Pseudomonas* increased significantly, but the relative abundance was below 50% at all sampling sites. Therefore, it can be inferred that the added ZS1 can adapt to the oil-contaminated environment and does not cause adverse effects on the indigenous environment, which once again proves that ZS1 has good application prospects in marine oil spill pollution remediation.

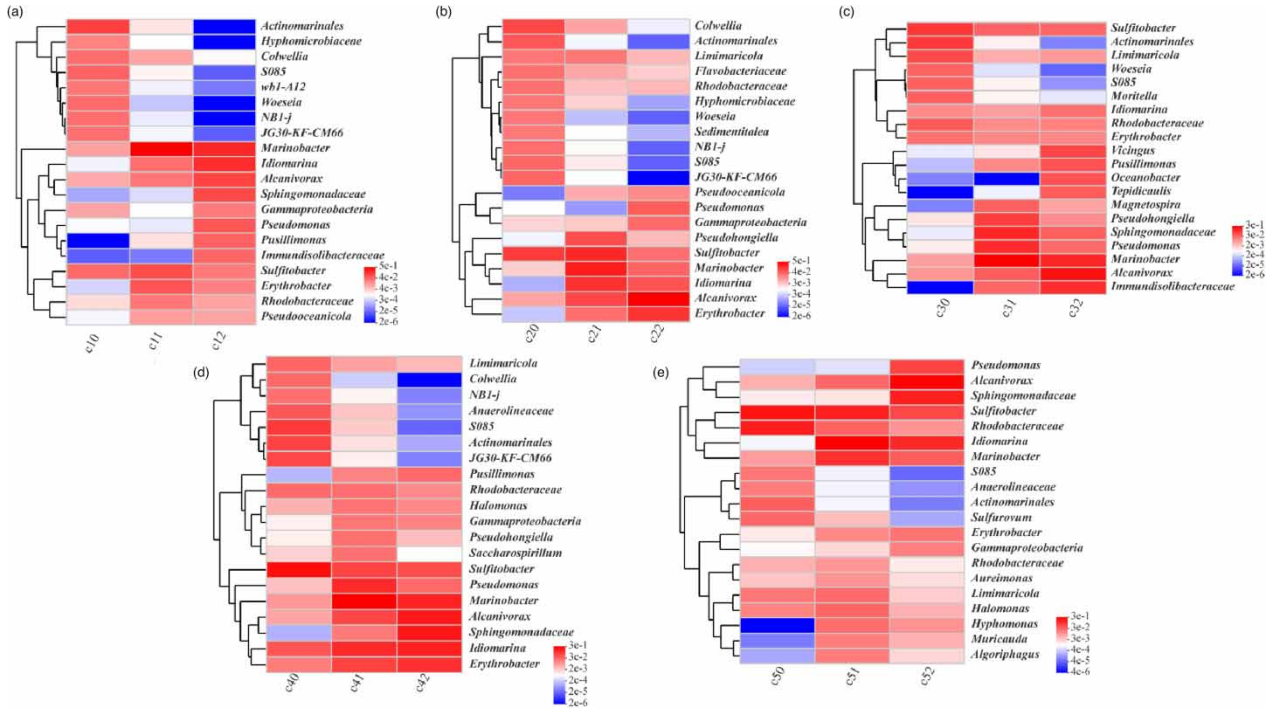


Figure 2 | Heat map of community structure at the genus level at each sampling site. The horizontal coordinates are the sample names and the vertical coordinates are the species names, the color gradients of the color blocks are used to show the changes in abundance of different species in the samples, and the values represented by the color gradients are shown on the right side of the figure. (a) Sample at QO2 sampling site; (b) sample at N3 sampling site; (c) sample at SO1 sampling site; (d) sample at SO3 sampling site; (e) sample at SO2 sampling site.

More specifically, it can be seen from Figure 2(a) that the percentage of strain abundance decreased from 59.3 to 22.3% in the water sample at QO2 point after bioremediation, although the strain *Marinobacter* remained as the dominant strain. At sampling point N3, after the occurrence of crude oil contamination, the dominant strain changed to *Marinobacter* (26.6%) and *Sulfitobacter* (19.8%), and the content of strain *Sulfitobacter* in the original water sample (C20) was 12.2%. After the addition of strain ZS1 for bioremediation, *Alcanivorax* (52.2%) and *Erythrobacter* (15.0%) became the new dominant strains. *Alcanivorax* which belongs to the family *Alcanivoracaceae* and class *Gammaproteobacteria* is a very common petroleum-degrading bacterium (Jagtap *et al.* 2021). Some studies have shown that due to the presence of several alkane hydroxylase systems in strain *Alcanivorax*, it can effectively degrade both branched and straight chain alkanes present in petroleum and is specialized for hydrocarbons, mainly aliphatic hydrocarbons (Joye *et al.* 2014; Uribe-Flores *et al.* 2019).

3.3. Effect of strain ZS1 on the degradation of crude oil

Residual crude oil in different microcosms was characterized by gas chromatography-mass spectrometry (GC-MS) after incubation for 50 days in Figure 3. The crude oil contained complex hydrocarbons, mainly long-chain and cyclic hydrocarbons compared to those available in the National Institute of Standards and Technology (NIST) library search system.

Figure 3(C1)–3(C2) show the GC-MS results of the samples without and with the addition of the bacterial solution. The sample without the addition of bacterial solution (Figure 3(C1)) has a higher peak, and it is obvious that the crude oil concentration of the sample with the addition of ZS1 bacterial solution is significantly lower.

From Figure 3, it was observed that the n-alkane carbon number of the crude oil used in this study lie in the range of C12–C21. Petroleum contains a wide variety of hydrocarbons, most of which can be degraded to different degrees by microorganisms (Chandran 2011). Generally speaking, alkanes in the range of C10–C18 are most easily degradable. The result showed that the efficient petroleum degrading bacteria ZS1 has different degradation effects on hydrocarbons with different chain lengths (Figure 3). ZS1 has a positive degradation effect on C18–C20, and the degradation rate of 50 d all reached more than 70%, in addition, the degradation rate of C13 reached 100% in 50 d. However, it was observed that the contents of C11, C16 and C17 in the degradation products were higher than the carbon content of the initial crude oil, which may be due to the breakage of long-chain alkanes into short-chain alkanes when they were degraded, resulting in the increase of C11, C16 and C17 alkanes. The results of this study are consistent with previous studies that *Pseudomonas aeruginosa* XJ16 biodegrades n-alkanes with relatively low carbon numbers more easily than n-alkanes with higher carbon numbers (Liu *et al.* 2020). It is remarkable that the presence of C40 and C44 was detected in the samples spiked with ZS1 bacterial solution, which may be the long chains formed after ring opening of aromatic hydrocarbons. It was mentioned in section 3.2 that *Marinobacter* was associated with aromatic hydrocarbon degradation and was present as the dominant strain in the samples spiked with ZS1 bacterial solution (Murphy *et al.* 2021). Strain ZS1 also contains aromatic hydrocarbon degradation genes and has some degradation effect on aromatic hydrocarbons. Both bacteria caused ring opening of aromatic hydrocarbons in the crude oil fraction to form long chains that were not present in the undegraded sample (Figure 3(C1)).

3.4. Analysis of the degradation pathway of strain ZS1

3.4.1. Whole genome sequencing of strain ZS1

Gene islands (GIs) are one of the most important forms of horizontal transfer elements that contain genes associated with multiple biological functions, and genes carried by GIs are often able to give bacteria a selective advantage. A total of 10 genomic islands were predicted in the genome of *Pseudomonas aeruginosa* ZS1 using Island Viewer (Table A3). Genes encoding proteins associated with the degradation of aromatic compounds were identified.

The genomic features of strain ZS1 were annotated and the results are shown in Figure 4. The average GC content of the ZS1 genome was 66.52%, while the N50 value of contigs was 372,673 bp and the gene length was 6,306,896 bp. The coding sequences in the genome (CDS) were predicted using software of Glimmer, GeneMarkS and Prodigal and 5990 coding genes, 60 tRNA genes and 3 rRNA genes were predicted. As shown in Figure A2, whole genome sequencing of strain ZS1 revealed a total of 2517 enzymes involved in metabolism or associated with metabolic functions. Among them, 108 genes were associated with lipid metabolism, 210 genes were associated with the metabolism of cofactors and vitamins, and 48 enzymes were involved in the metabolism of terpenoids and polyketides. This provides a solid database for studying the degradation pathway of crude oil by strain ZS1.

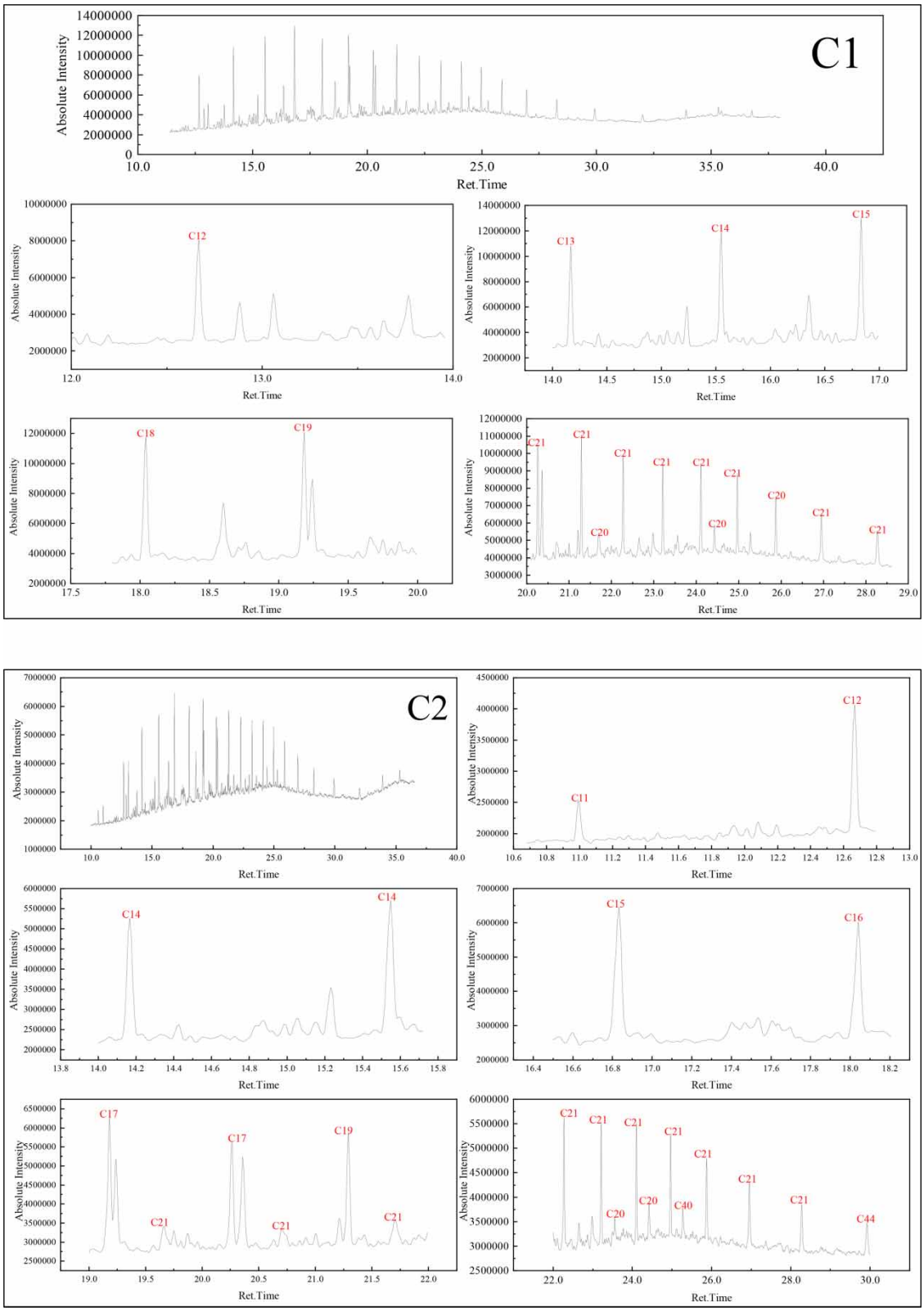


Figure 3 | GC-MS characterization of residual crude oil at 50 d. (C1) Addition of crude oil; (C2) Degradation of crude oil by added crude oil and activated ZS1 bacteria.

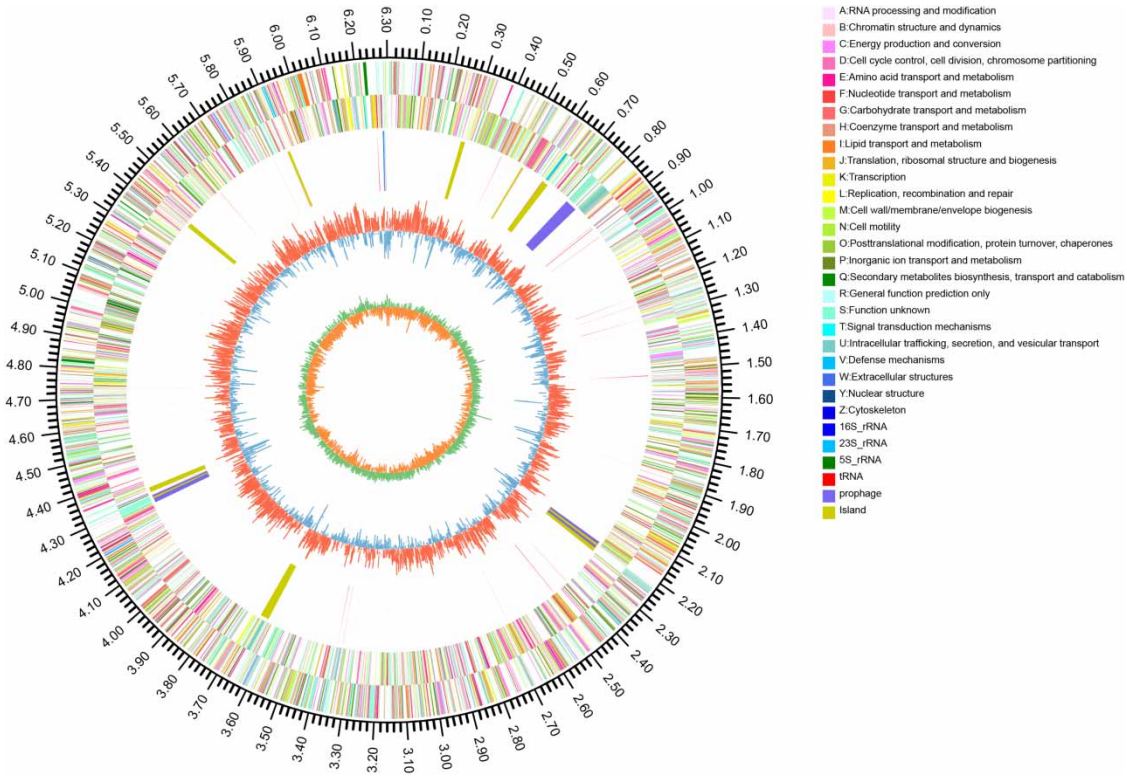


Figure 4 | Circos genome circle diagram. The outermost circle of the circle diagram is the identification of genome size; the second and third circles are the CDS on positive and negative strands, and different colors indicate the functional classification of different COGs of CDS; the fourth circle is the rRNA and tRNA; the fifth circle is the GC content; the innermost circle is the GC-Skew value, and the specific algorithm is G–C/G + C.

3.4.2. Degradation of n-alkanes

The n-alkanes are generally degraded by terminal oxidation or secondary oxidation pathways (Rojo 2009; Hong *et al.* 2016, 2017). In the terminal oxidation pathway, alkane degradation is initiated by the oxidation of terminal methyl groups to produce primary alcohols, which are converted to the corresponding aldehydes and further oxidized to fatty acids (Jimenez *et al.* 2002; Hong *et al.* 2017). Subsequently, the fatty acids bind to coenzyme A and undergo *β*-oxidation to produce acetyl coenzyme A. Acetyl coenzyme A is further processed through the trichloroacetic acid cycle to eventually produce H_2O and CO_2.

After whole genome sequencing of *Pseudomonas aeruginosa* ZS1, some enzymes involved in the above process were identified, such as monooxygenase (van Beilen & Funhoff 2007), dioxygenase, acyl coenzyme synthase, dehydrogenase, etc. As shown in Figure 5(I), alkB (alkane monooxygenase; gene1512) together with ladA (Dimethyl-sulfide monooxygenase; gene4918, gene4942) act together with n-alkanes, causing the oxidation of the terminal methyl group of the alkane to a primary alcohol (R-CH2-OH) (Das *et al.* 2015). Among them, *ladA* belongs to the bacterial luciferase family (Hong *et al.* 2017) and plays an important role in the initial oxidation of C15–C36 alkanes. This is followed by the conversion of primary alcohols to fatty acids by the action of alcohol dehydrogenases (gene280, gene589, gene2055, etc. Table A4) and aldehyde dehydrogenases (gene167, gene714, gene5741, etc. Table A5). ZS1 contains acyl coenzyme A synthase (gene1137, gene5503), which can catalyze the binding of fatty acids to coenzyme A. It then enters the *β*-oxidation process to generate acetyl coenzyme A to enter the TCA cycle process. It was identified that ZS1 lacks *Baeyer-Villiger monooxygenase*, an essential enzyme involved in cycloalkane degradation and secondary oxidation of n-alkanes (Gregson *et al.* 2018). Therefore, it was proposed that ZS1 cannot degrade cycloalkanes and there is no secondary oxidation pathway.

3.4.3. Degradation of aromatic compounds

Microorganisms accomplish the degradation and mineralization of aromatic hydrocarbons mainly under aerobic conditions (Seo *et al.* 2009; Li *et al.* 2019). In the process of the hydroxylation and cleavage of aromatic compounds, microorganisms

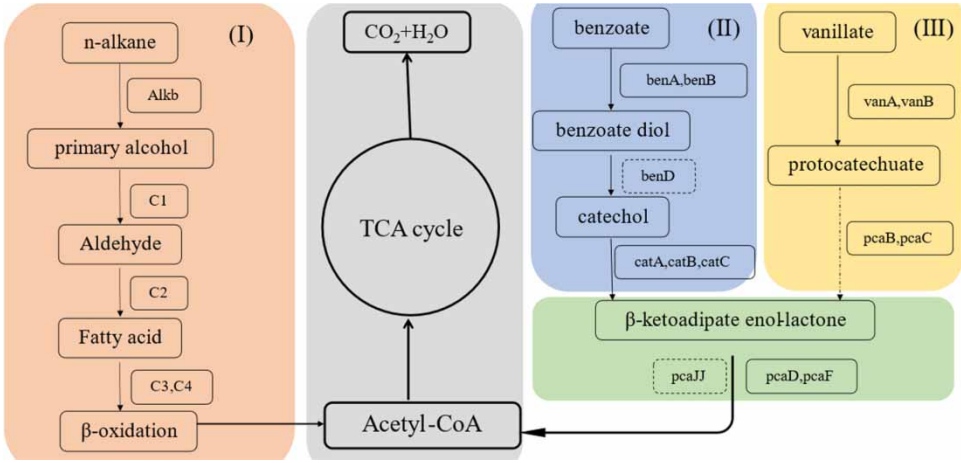


Figure 5 | Degradation pathway diagram of n-alkane and aromatic hydrocarbon decomposition in *Pseudomonas aeruginosa* and its connection with the tricarboxylic acid cycle. C1, alcohol dehydrogenase; C2, aldehyde dehydrogenase; C3, acyl-CoA synthetase; C4, fatty acid hydroxylase (I) Degradation pathway of n-alkanes of ZS1; (II) Catechol pathway; (III) Protocatechuic acid pathway.

produce mono- or di-oxygenase, which converts aromatic compounds into central intermediates such as catechol (1,2-dihydroxybenzene), protocatechuate (3,4-dihydroxybenzoate) and gentisate (2,5-dihydroxybenzoate) (Seo *et al.* 2009). These intermediates are cleaved by dioxygenases and undergo adjacent or interposition cleavage. The products of the adjacent pathway (acetyl coenzyme A and succinyl coenzyme A) and the coenzyme pathway (pyruvate and acetaldehyde) enter the trichlorohydric acid cycle (Das *et al.* 2020). The protocatechuic acid and catechol pathways are common degradation pathways for aromatic compounds.

In the catechol pathway (Figure 5(II)), phthalates are changed to benzoate diol (benezoate diol) by *benA* (benzoate 1,2-dioxygenase; gene1578), *benB* (benzoate 1,2-dioxygenase; gene1579) and then by *benD* (not identified) to catechol. The catechol is formed by the action of *catA* (catechol 1,2-dioxygenase; gene1591), *catB* (type B chloramphenicol acetyltransferase; gene788, gene1589), *catC* (muconolactone Delta-isomerase; gene1590) in turn to produce *β*-ketoadipate enolactone. As for the protocatechuic acid pathway (Figure 5(III)), ZS1 contains *vanA* (vanillate monooxygenase; gene2589) and *vanB* (vanillate monooxygenase ferredoxin subunit; gene2590). *VanB* plays an important role in the production of protocatechuic acid salts from vanillate. It is followed by *pcaB* (3-carboxy-cis, cis-muconate cycloisomerase; gene4734) and *pcaC* (4-carboxymuconolactone decarboxylase; gene4732) to produce *β*-ketoadipate lactone (Medić *et al.* 2022). Both of the above pathways finally produce *β*-ketoadipate enol-lactone. Then acetyl coenzyme A and succinyl coenzyme A were generated by the action of *pcaD* (3-oxoadipate enol-lactonase; gene2983, gene4733), *PcaIJ* (not identified), and *pcaF* (3-oxoadipyl-CoA thiolase; gene4736) (Hong *et al.* 2017). Finally, it enters the TCA cycle for further metabolism. In addition, the homogentisate pathway is proved to exist in the degradation of crude oil. It was found that *phhA* (phenylalanine 4-monooxygenase, gene577) and *phhB* (Pterin-4-alpha-carbinolamine dehydratase, gene578) play an important role in the conversion of phenylalanine to tyrosine (Donoso *et al.* 2021). The sequencing results showed that strain ZS1 contained both genes. Tyrosine is catalyzed by *tyrB* (tyrosine aminotransferase; gene579, gene865) to produce 4-hydroxyphenylpyruvate. It is finally converted to homogentisate. The catechol pathway is the most complete compared to the above three pathways.

4. CONCLUSION

In this study, the impact of oil spill pollution on deep-sea indigenous microorganisms in the South China Sea and their response, as well as the role of petroleum degrading bacteria in the remediation process and the degradation mechanism, were discussed. The results showed that *Pseudomonas aeruginosa* ZS1 could significantly promote the degradation of crude oil in seawater. The degradation rate increased by up to 43.44% compared with the samples without ZS1 addition. In addition, crude oil contamination could seriously affect the composition of microbial communities in seawater, the relative abundance of all sampling sites decreased after adding crude oil, and the largest changes in *Marinobacter* (59.3 to 22.3%) occurring at the N3 sampling site. The dominant microorganisms changed from *Actinomarinales* and *Sulfitbacter* in the original seawater to *Marinobacter*, *Alcanivorax* and *Idiomarina*. ZS1 degraded n-alkanes through the terminal oxidation pathway,

and the degradation of aromatic compounds was achieved mainly by the catechol pathway. According to the current research results, it is known that the bioremediation cycle of marine oil spills is long, and methods such as injection of bacteriological agents can be considered to shorten the remediation cycle. Meanwhile, the actual ocean conditions should be simulated as much as possible, and the effects of environmental factors such as temperature, dissolved oxygen and pressure on oil biodegradation should be considered comprehensively.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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