The complete genomic sequence of a novel cold-adapted bacterium, *Planococcus maritimus* Y42, isolated from crude oil-contaminated soil

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Abstract

*Planococcus maritimus* Y42, isolated from the petroleum-contaminated soil of the Qaidam Basin, can use crude oil as its sole source of carbon and energy at 20 °C. The genome of *P. maritimus* strain Y42 has been sequenced to provide information on its properties. Genomic analysis shows that the genome of strain Y42 contains one circular DNA chromosome with a size of 3,718,896 bp and a GC content of 48.8%, and three plasmids (329,482; 89,073; and 12,282 bp). Although the strain Y42 did not show a remarkably higher ability in degrading crude oil than other oil-degrading bacteria, the existence of strain Y42 played a significant role to reducing the overall environmental impact as an indigenous oil-degrading bacterium. In addition, genome annotation revealed that strain Y42 has many genes responsible for hydrocarbon degradation. Structural features of the genomes might provide a competitive edge for *P. maritimus* strain Y42 to survive in oil-polluted environments and be worthy of further study in oil degradation for the recovery of crude oil-polluted environments.

Keywords: *Planococcus maritimus*, Qaidam Basin, Crude oil, Degradation, Genome

Introduction

Oil spills occur frequently and pose a severe hazard to pristine ecological conditions [1, 2]. On account of the difficulty in degrading crude oil, the pollutant remains in the environment to contaminate ground water and air, affect crop growth and endanger human health [3, 4]. Bioremediation is currently recognized as the preferred strategy to utilize biological activities to rapidly eliminate hydrocarbon pollutants [5]. Many microorganisms, especially bacteria, have been found to participate in the process of biodegradation in contaminated environments [6, 7].

*Planococcus*, as a psychrotolerant and halotolerant bacterium, was also reported as having the ability to degrade crude oil [8–10]. For example, a cultured *Planococcus* sp. strain S5 was described to be able to grow on salicylate or benzoate [11], and *Planococcus alkanoclasticus* was capable of degrading linear alkanes [9]. Meanwhile, most of the *Planococcus* bacteria have showed the ability to withstand heavy metals, produce surfactants and adapt to cold and/or saline environments [12–14]. Because of the above properties, *Planococcus* exhibited a potential capability in the bioremediation of extremely contaminated environments. Although many studies have reported the genomic backgrounds of *Planococcus* strains, oil biodegradation mechanisms in *Planococcus* have rarely been discussed. In the present study, we isolated a *Planococcus* strain from the oil-contaminated soils in the Qinghai-Tibetan Plateau. Our aims were to characterize the genome of this oil-degrading strain and to further seek responsible strategies associated with oil degradation in low-temperature environments.
Organism information
Classification and features

In this experiment, a novel cold-adapted strain Y42 was isolated from oil-contaminated soils in the Lenghu oil field, which is located in the northern margin of the Qaidam Basin (93.34°E, 38.71°N). The molecular identification of the strain was performed using the primers 27F and 1492R to amplify and sequence the 16S rRNA gene [15]. Phylogenetic analysis based on 16S rRNA gene sequence similarity showed that strain Y42 was closely related to members of the genus *Planococcus* (*Planococcus maritimus* (97%)). The strain Y42 was thus recognized as a potential new member of *Planococcus* (Fig. 1).

The strain Y42 was able to grow at moderately low temperatures, and many members of the genus *Planococcus* had been predominantly isolated from frozen and/or saline environments [16]. Cell micrographs were obtained by using a scanning electron microscope (SEM) on cells grown in LB medium. Cells of strain Y42 were coccoid, typically 0.7–1 μm in diameter, and diplococci were observed, along with cell division septa (Fig. 2a). Colony morphology was determined on LB plates following 3–5 days of growth at 25 °C, which resulted in the formation of orange, round, umbonate colonies (Fig. 2b). Additional characteristics of *P. maritimus* Y42 are shown in Table 1.

Crude oil-degrading characterization of strain Y42 was completed under specified growth conditions with crude oil as the sole carbon source by using a gas chromatography-mass spectrometry (GC-MS) method. The strain Y42 was cultured with MM medium (3.5 g of MgCl₂, 1.0 g of NH₄NO₃, 0.35 g of KCl, 0.05 g of CaCl₂, 1.0 g of KH₂PO₄, 1.0 g of K₂HPO₄, 0.01 g of FeCl₃, 0.08 g of KBr, and 24 mg of SrCl₂·6H₂O, pH 7.5) with crude oil as a carbon source and incubated at 20 °C for 10 d [17]. A parallel experiment without inoculation was used as the control. The remaining oil from the cultures was extracted with 15 mL of hexane in a separating funnel at room temperature, and anhydrous Na₂SO₄ was then added to remove residual water. Ultimately, the extracted oil was analysed using a GC-MS method [18]. For GC-MS analysis, one microliter of the filtered solution was injected into a quartz capillary column (DB-WAX, 30 m × 0.25 mm × 0.25 μm). The total area of a detected individual hydrocarbon peak was defined as its hydrocarbon concentration in crude oil. The degradation rate of the components of crude oil was determined according to the following equation: \[ \eta = \left(1 - \frac{n_1}{n_2}\right) \times 100\% \], where \( \eta \), \( n_1 \), and \( n_2 \) are the degradation rate of the components of crude oil, the peak area of the components of crude oil remaining in the samples, and the peak area of the components of crude oil in the controls, respectively [19]. The chromatograms revealed that the concentrations of the components of crude oil, including \( n \)-alkanes, branched alkanes, cyclanes, and aromatic hydrocarbons, were lower in the sample treated with the strain...
**Table 1** Classification and general features of *P. maritimus* Y42

| MIGS ID | Property            | Term                        | Evidence code |
|---------|---------------------|-----------------------------|---------------|
|         | Classification      | Domain Bacteria             | TAS [42]      |
|         | Phylum              | Firmicutes                  | TAS [43]      |
|         | Class               | Bacilli                      | TAS [44, 45]  |
|         | Order               | Bacillales                   | TAS [46, 47]  |
|         | Family              | Planococcaceae               | TAS [46, 48]  |
|         | Genus               | Planococcus                  | TAS [46, 49]  |
|         | Species             | Planococcus Strain Y42       | TA            |
|         | Gram stain          | Positive                     | TA            |
|         | Cell shape          | Coccoid                      | TA            |
|         | Motility            | Motile                       | TA            |
|         | Sporulation         | Non-sporulating              | TA            |
|         | Temperature range   | 4–30 °C                      | TA            |
|         | Optimum temperature | 25 °C                        | TA            |
|         | pH range; Optimum   | 6–9; 7.5;                    | TA            |
|         | Carbon source       | Yeast extract                | TA            |
| MIGS-6  | Habitat             | Frozen soil                  | TA            |
| MIGS-6.3| Salinity            | < 15% NaCl (w/v)             | TAS [50]      |
| MIGS-22 | Oxygen requirement  | Aerobic                      | NA           |
| MIGS-15 | Biotic relationship | Free-living                  | TA            |
| MIGS-14 | Pathogenicity       | Non-pathogen                 | NA           |
| MIGS-4  | Geographic location | China: Qaidam Basin, Lenghu area | TA          |
| MIGS-5  | Sample collection   | 2015                         | TA            |
| MIGS-4.1| Latitude            | + 38.71 (38°43′10.11″)       | NA           |
| MIGS-4.2| Longitude           | + 93.34 (93°20′30.1″)        | NA           |
| MIGS-4.4| Altitude            | 2789 m                       | NA           |

*Evidence codes — IDA Inferred from Direct Assay, TAS Traceable Author Statement (i.e., a direct report exists in the literature), NAS Non-traceable. Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project.*

*P. maritimus* Y42 than the abiotic control sample (Fig. 3a). After incubation for 10 days at 20 °C, the preferred degradation occurred in short-chain *n*-alkanes ranging from C12 to C18, C12 was particularly decomposed, by approximately 50%. Meanwhile, the other straight-chain alkanes and aromatic hydrocarbons were decomposed by 20–30% (Fig. 3b). The strain Y42 did not show a remarkably higher ability to degrade different components of crude oil than other strains such as *Bacillus* [20, 21], *Pseudomonas* [22, 23], *Rhodococcus* [24], and etceteras. Even so, as an indigenous oil-degrading bacterium, the existence of the *P. maritimus* strain Y42 played a significant role in reducing overall environmental impact of the oil [25] and greatly enriched microbial community structures in the oil-contaminated soils in low-temperature environments [26].

**Genome sequencing information**

**Genome project history**

This organism was selected for sequencing based on its phylogenetic position and its ability to degrade crude oil. The genome project was deposited in the genome online database [27] and the complete genome sequence was available in GenBank (NCBI-Genome). Sequencing,
finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information was provided in Table 2.

Growth conditions and genomic DNA preparation

*P. maritimus* strain Y42 was inoculated into LB liquid medium and grown on a gyratory shaker (200 rpm) at 20 °C for 96 h. Genomic DNA of the strain was extracted using the Bacterial Genomic DNA Extraction Kit (AxyPrep) as per its operation instruction.

Genome sequencing and assembly

The complete genome sequence of *P. maritimus* strain Y42 was generated by combined Illumina MiSeq with PacBio platform [28]. The reads generated with Illumina MiSeq platform were de novo assembled using Hierarchical Genome Assembly Process (HGAP) [29]. Gaps between contigs were closed by using the SPAdes-3.5.0. This whole genome project (Bioproject ID: PRJNA371518) has been registered and assembled sequence data submitted at NCBI GenBank under the accession no. CP019640.1-CP019643.1. And this finished genome was deposited in IMG database with the Project ID: Gp0209326.

Genome annotation

The completed genomic sequence was predicted using the Glimmer software 3.0 [30]. tRNA genes were predicted using tRNAscan-SE 1.3.1 [31] and rRNA genes were identified using Barrnap 0.4.2 [32]. The rest of the non-coding rRNA genes were predicted by using BLASTp against databases NCBI-NR database (http://www.ncbi.nlm.nih.gov/) and genes function annotations were assigned by the COG database (http://www.ncbi.nlm.nih.gov/COG/).

Genome properties

The assembled genome of *P. maritimus* Y42 consisted of one circular DNA chromosome with a size of 3,718,896 bp and a GC content of 48.8% and three plasmids (329,482; 89,073; and 12,282 bp) (Table 3). Genome project information and genomic features are summarized in Table 4. From a total of 4155 genes, 3947 were annotated as predicted protein-coding sequences (CDS). In addition, the genome included 70 tRNA genes, 27 rRNA genes, 4 ncRNA genes, and 108 pseudogenes.

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### Table 2 Project information of the whole genome sequence of *P. maritimus* Y42

| MIGS ID | Property               | Term               |
|---------|------------------------|--------------------|
| MIGS-31 | Finishing quality      | Finished           |
| MIGS-28 | Libraries used         | Paired-end and PacBio |
| MIGS-29 | Sequencing platforms   | Illumina HiSeq 2000 and PacBio |
| MIGS-31.2 | Fold coverage     | PacBio: 300x       |
| MIGS-30 | Assemblers            | SPAdes v. 3.5.0, HGAP |
| MIGS-32 | Gene calling method   | Glimmer 3.02       |
| Locus Tag |                      | BOX71              |
| GenBank ID |                    | CP019640.1-CP019643.1 |
| GenBank Date of Release |            | April 14, 2017   |
| GOLD ID  |                      | Gp0209326          |
| BIOPROJECT |                  | PRJNA371518       |
| MIGS-13  | Source Material Identifier | Y42            |
| Project relevance |                | Biodegrading      |

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### Table 3 Summary of genome: 1 chromosome and 3 plasmids

| Label   | Size (Mb) | GC%  | INSDC identifier | RefSeq ID       |
|---------|-----------|------|------------------|-----------------|
| Chromosome | 3.72     | 48.8 | CP019640.1       | NZ_CP019640.1   |
| Plasmid 1 | 0.329482  | 44.8 | CP019641.1       | NZ_CP019641.1   |
| Plasmid 2 | 0.089073  | 43.6 | CP019642.1       | NZ_CP019642.1   |
| Plasmid 3 | 0.012282  | 45   | CP019643.1       | NZ_CP019643.1   |
Open reading frames (ORFs) were assigned into 23 functional categories under the Clusters of Orthologous Groups (COGs) and are represented in a circular genome map in Fig. 4. The COG distribution of genes is shown in Table 5. The genome map was visualized by the CG View server.

**Insights from the genome sequence**

Genome annotation predicted that many genes support the adaptability of strain Y42 to cold and crude oil-contaminated environments. Based on the COG analysis, the genes related to general function prediction only (R) and amino acid transport and metabolism (E) were relatively enriched over the other functional genes. The results indicate genome-wide selection pressure [33]. Moreover, the abundance of genes related to functions unknown (S) in strain Y42 suggested that the strain may possess many new genes.

Further analysis showed that many key oxygenase genes were located in the *P. maritimus* Y42 genome, including those of catechol 1,2-dioxygenase (*catA*),

| Attribute                        | Value     | % of Total |
|----------------------------------|-----------|------------|
| Genome size (bp)                 | 4,149,733 | 100        |
| DNA coding (bp)                  | 3,541,381 | 85.34      |
| DNA G+C (bp)                     | 2,005,184 | 48.32      |
| DNA scaffolds                     | 4         | 100        |
| Total genes                      | 4283      | 100        |
| Protein coding genes             | 4172      | 97.41      |
| RNA genes                        | 111       | 2.59       |
| Pseudo genes                     | 108       |            |
| Genes in internal clusters       | NA        |            |
| Genes with function prediction   | 3162      | 73.83      |
| Genes assigned to COGs           | 2696      | 62.95      |
| Genes with Pfam domains          | 3323      | 77.59      |
| Genes with signal peptides       | 186       | 4.34       |
| Genes with transmembrane helices | 959       | 22.39      |
| CRISPR repeats                   | NA        |            |

**Table 4 Genomic statistics of *P. maritimus* Y42**
catechol 2,3-dioxygenase (catE), and cytochromes P450. In addition, dehalogenase-coding genes were also found in the chromosome; these genes were involved in numerous metabolic processes such as the degradation of chlorocyclohexane, chlorobenzene, chloroalkane and chloroalkene [34]. A total of 9 genes putatively encoding for crude oil metabolites were identified in this genome (Fig. 5). The existence of these oxygenase genes could regioselectively oxidize substrates, especially natural aromatic compounds, by transferring oxygen to the substrates and transforming non-reactive hydrocarbons into available hydrocarbons [35, 36]. However, genes responsible for n-alkane degradation, such as the alkB gene, which is considered as functional biomarker gene for alkane degradation [37–39], were not found in the genome of strain Y42. These results imply that the strain Y42 might have some novel genes that participate in the catabolism of n-alkane pollutants.

In addition, three cold shock proteins (WP_008296927.1, WP_026692369.1, WP_008298364.1) were predicted, and these proteins were supposed to play important roles under low-temperature conditions [40]. In total, 238 genes were predicted to be involved in transport systems for aromatic compounds, amino acids, carbohydrates, lipids and inorganic ions. Among these genes, several osmoprotectant transport system (Opu) genes were identified to likely maintain the homeostasis of strain Y42. Furthermore, a large number of divalent cation transport and sulfate/phosphonate/nitrogen uptake systems guarantee the supply of nutrient elements for microbes in crude oil environments [41]. These genes were essential for strain Y42 to gain a competitive edge in oil-polluted soils.

Conclusions

The strain Y42, as a potential new member of Planococcus, was isolated from a cold and crude oil-contaminated

| Abb. | Function | From | To | Size (bp) |
|------|----------|------|----|-----------|
| catA | catechol 1,2-dioxygenase | 3365427 | 3366194 | 768 |
| catE | catechol 2,3-dioxygenase | 3211222 | 3212070 | 849 |
| catB | muconate lyase | 3359565 | 3360704 | 1140 |
| divA | haloalkane dehalogenase | 2003708 | 2004586 | 879 |
| L-DEX | 2-bromoacetate dehalogenase | 909469 | 910176 | 708 |
| frm | 4-formylmaleate reductase | 3056703 | 3057842 | 1140 |
| ALDH | aldehyde dehydrogenase | 1240609 | 1242135 | 1527 |
| CYP102 | cytochrome P450 | 2726711 | 2729905 | 3195 |
| porC | cytochrome | 3000906 | 3001091 | 186 |

Fig. 5 Gene clusters in the genome of P. maritimius strain Y42 encoding metabolic functions for oil degradation. The corresponding oil degradation related genes are red colored.

Table 5 Number of genes of P. maritimius Y42 with the general COG functional categories

| Code | Value | % of total | Description |
|------|-------|------------|-------------|
| J    | 225   | 7.34       | Translation, ribosomal structure and biogenesis |
| A    | 0     | 0          | RNA processing and modification |
| K    | 185   | 6.04       | Transcription |
| L    | 117   | 3.82       | Replication, recombination and repair |
| B    | 1     | 0.03       | Chromatin structure and dynamics |
| D    | 36    | 1.17       | Cell cycle control, Cell division, chromosome partitioning |
| V    | 71    | 2.32       | Defense mechanisms |
| T    | 144   | 4.7        | Signal transduction mechanisms |
| M    | 134   | 4.37       | Cell wall/membrane biogenesis |
| N    | 47    | 1.53       | Cell motility |
| U    | 33    | 1.08       | Intracellular trafficking and secretion |
| O    | 118   | 3.85       | Posttranslational modification, protein turnover, chaperones |
| C    | 183   | 5.97       | Energy production and conversion |
| G    | 172   | 5.61       | Carbohydrate transport and metabolism |
| E    | 297   | 9.69       | Amino acid transport and metabolism |
| F    | 95    | 3.1        | Nucleotide transport and metabolism |
| H    | 161   | 5.25       | Coenzyme transport and metabolism |
| I    | 172   | 5.61       | Lipid transport and metabolism |
| P    | 187   | 6.1        | Inorganic ion transport and metabolism |
| Q    | 95    | 3.1        | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 325   | 10.6       | General function prediction only |
| S    | 180   | 5.87       | Function unknown |
| –    | 1587  | 37.05      | Not in COGs |

*The total is based on the total number of protein coding genes in the genome.
environment. A genomic analysis of strain Y42 provided the theoretical basis for the mechanism of oil degradation by bacteria. Genes involved in cold shock and transport systems point to the potential capacity of strain Y42 for soil bioremediation contaminated by aromatic compounds in cold environments. Genomic research on strain Y42 would also provide a blueprint for the application of bioremediation and recovery in cold oil-polluted environments.

Abbreviation
CDSS: protein-coding sequences; COG: Clusters of Orthologous Groups categories; GC-MS: gas chromatography and mass spectrometric Detector; ORFs: open reading frames

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Authors’ contributions
RQY, WZ and GSZ initiated the study. GSZ, TC and GXL designed the research and project outline. RQY drafted the manuscript. RQY and SJC isolated the strain. RQY assembled and annotated the genome. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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