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Complete genome sequence of *Alcanivorax xenomutans* P40, an alkane-degrading bacterium isolated from deep seawater

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ABSTRACT

Strain P40 (MCCC 1A01128) is a member of *Alcanivorax xenomutans*, in the family *Alcanivoracaceae* of *Gammaproteobacteria*. Since *Alcanivorax* species play a pivotal role in bioremediation of oil spills in marine environments, further studies on these hydrocarbonoclastic marine bacteria will facilitate a better understanding of their alkane metabolic capacities. Previous study shows strain P40 has obvious ability of alkane degradation. Here, we describe the complete genome sequence and annotation of strain P40, which is the first strain with the complete genome sequence of the species *A. xenomutans*. Strain P40 contains a 4,733,951 bp chromosome without any plasmids, and encodes 4148 protein-coding genes and 45 RNA-only encoding genes. With genes involving in alkane degradation, heavy-metal resistance, stress response and so on, *A. xenomutans* P40 may have a potential use in the bioremediation of oil polluted and heavy metal-contaminated environments.

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1. Introduction

Nowadays, oil spills are still a central source of ocean pollution, threating marine ecosystems. Fortunately, the activities of naturally microbes have the ability of eliminating those considerable amounts of petroleum entering the sea (Timmis, 2010). Since *Alcanivorax* species can use alkanes more effectively than other hydrocarbon-degrading bacteria (Hara et al., 2003; Liu et al., 2011), thus becoming the most important alkane-degrading bacteria in marine environments. The genus *Alcanivorax* belongs to family *Alcanivoracaceae* of *Gammaproteobacteria*, and currently comprises eleven species including *A. borkumensis* (Yakimov et al., 1998), *A. jadensis* (Bruns and Berthe-Corti, 1999), *A. venustensis* (Fernández-Martínez et al., 2003), *A. dieselolei* (Liu and Shao, 2005), *A. balearicus* (Rivas et al., 2007), *A. hongdengensis* (Wu et al., 2009), *A. pacificus* (Lai et al., 2011), *A. marinus* (Lai et al., 2013), *A. xenomutans* (Rahul et al., 2014) and *A. gelatiniphagus* (Kwon et al., 2015), *A. nanhaiticus* (Lai et al., 2016).

Strain P40 was isolated from the deep seawater of the Indian Ocean during Chinese Global Ocean Expedition of the "the R/V Dayang Yihao" in 2005 after enriched with petroleum and diesel as carbon source. The 16S rRNA gene shared 99.8%, 99.4% and 99.4% similarities with *Alcanivorax dieselolei* B-5^T, *Alcanivorax xenomutans* JC109^T and *Alcanivorax balearicus* MACL04^T, respectively. And the *gyrB* gene sequence of strain P40 shared the highest similarity with *Alcanivorax xenomutans* JC109^T (99.7%), followed by *Alcanivorax dieselolei* B-5^T (84.5%) and *Alcanivorax balearicus* MACL04^T (83.3%), and other species of genus *Alcanivorax* (70.9%–77.5%),thus indicating strain P40 belonged to the species *Alcanivorax xenomutans*. Based on its phylogenetic position and dominance position in the crude oil-degrading, we selected this organism for sequencing. In this report, we present the genome sequence and annotation of *A. xenomutans* P40, which is the first strain with the complete genome sequence of the species *A. xenomutans*. Its genome sequence and its curated annotation will provide significant datasets to better understand the physiology and metabolic potential of *A. xenomutans* and will greatly facilitate functional genomic studies of *A. xenomutans*.

2. Data description

General features of strain P40 are summarized in Table 1. The strain is positive for catalase, oxidase (weak), nitrate reduction, and negative for denitrification, arginine dihydrolase, indole production, D-glucose fermentation, urease, β-glucosidase, gelatin hydrolysis, β-galactosidase,

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Table 1
General features of *Alcanivorax xenomutans* P40 and MIGS mandatory information.

Items	Description
General features	
Classification	Domain *Bacteria* Phylum Proteobacteria Class *Gammaproteobacteria* Order *Oceanospirillales* Family *Oceanospirillales* Genus *Alcanivorax* Species *Alcanivorax xenomutans*
Gram stain	Negative
Cell shape	Rod
Motility	Motile
Pigmentation	No-pigmented
Sporulation	Non-sporulating
Temperature range	4–43 °C
Optimum temperature	28 °C
Carbon source	Sodium acetate, alkanes
Energy source	Chemoorganotrophic
Terminal electron receptor	Oxygen
Salinity	0.5–15%
Oxygen	Aerobic
MIGS data	
Submitted to INSDC	GenBank (ID: CP012331)
Investigation type	Bacteria
Project name	*Alcanivorax xenomutans* strain: P40 genome sequencing
Geographic location (latitude and longitude)	25.32°S, 70.04°E
Geographic location (depth)	−668 m
Geographic location (country)	Indian Ocean
Collection date	2005
Environment (biome)	Ocean
Environment (feature)	Water
Environment (material)	Sea water
Environmental package	Deep sea water samples from Indian Ocean
Biotic relationship	Free-living
Pathogenicity	None
Sequencing method	Illumina Hiseq2000, 454 FLX +
Assembly	GS *De Novo* Assembler package
Finishing strategy	Complete

D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate. API ZYM test strip results indicate that it is positive for alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine aminopeptidase, naphtol-AS-Bl-phosphoamidase, valine aminopeptidase (weak); negative for cystine aminopeptidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase. The API 20NE test strip shows that strain P40 can utilize adipic acid, capric acid, phenylacetic acid, N-acetyl-glucosamine (weak), trisodium citrate (weak), cannot utilize malic acid.

For genome sequencing, strain P40 was then grown aerobically to mid logarithmic phase at 28 °C in 216 L medium (containing, per litre seawater: CH_3COONa, 1.0 g; tryptone, 10.0 g; yeast extract, 2.0 g; sodium citrate, 0.5 g; NH_4NO_3, 0.2 g; pH 7.5). The genomic DNA was then extracted, concentrated and purified using the AxyPrep bacterial genomic DNA mini-prep Kit (Axygen). The purity and quality of DNA (UV A_{260}/A_{280}) was assessed using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA). The whole-genomic DNA sequencing was performed using a combination of Illumina and Roche454 platforms. The pair-end 454 library with average insert size of 3 kb pyrosequencing generated 543,410 reads totaling 233.4 Mb. GS *De Novo* Assembler package (v2.8) was then applied for assembly to obtain the draft genome scaffolds. Then Illumina Hiseq 2000 pair-end reads with average insert size of both 300 bp and 500 bp were realigned to the scaffolds to correct potential base errors and to fill the intra-scaffold gaps. The inter-scaffold gaps were filled in by sequencing the PCR products using ABI 3730xl capillary sequencers. Finally, sequences were assembled and assessed quality using Phred/Phrap/Consed software packages.

Circos software was then used to get circularized map of the chromosome, setting calculation window as 2000 bp and steps as 500 bp (Krzywinski et al., 2009). Gene prediction and annotation were performed using National Center for Biotechnology Information (NCBI) prokaryotic genome annotation pipeline (Tatusova et al., 2016) and the Rapid Annotation using Subsystem Technology (RAST) pipeline (http://rast.nmpdr.org/) (Overbeek et al., 2014). The functional annotation of predicted ORFs was used to search the KEGG and COG databases by RPS-BLAST.

The complete genome consists of one chromosome with a total length of 4,733,951 base pairs (bp) and a G + C content of 61.45% (Fig. 1). Of the 4341 genes predicted, 4148 were protein-coding genes (coding sequence, CDS), and 45 RNAs; 143 pseudogenes were also identified. Of the entire 4148 CDS, 2092 CDS were identified to participate in 223 pathways. In addition, 3618 could be assigned to cluster of orthologous groups (COGs), which were analyzed to understand how strain P40 deploys its genes in the genome. These CDS could be assigned to 25 different categories (Table 2).

28 putative Genomic islands (GIs) were identified by IslandViewer (Langille and Brinkman, 2009) (Table S1). The size of the 28 putative islands ranged from 3721 bp (GI 3) to 3,9969 bp (GI 21). The largest GI 21 contained 32 genes, whereas the smallest GI 3 had 6 genes. In these 28 GIs, 234 CDS were identified, including CDS encoding regulators, transmembrane proteins, heavy metal ion transport and resistance related proteins, CRISPR-associated protein for defense, oxidoreductases for metabolism and so on. Among these GIs, eight contain mobile genetic elements, such as integrase and transposase genes, suggesting that these GIs can self-mobilize and could also support potential active horizontal gene transfer in the strain.

To further understanding adaptive capacity of strain P40 to marine environment, metabolic features related to functional categories were then analyzed (Table S2). 125 genes were found relating to "Virulence, Disease and Defense" which functioned as antibiotics and toxic compounds resistance, invasion and intracellular resistance and bacteriocins synthesis. 72% genes belonged to the subcategory of resistance to antibiotics and toxic compounds, and majority involving in heavy-metal resistance, such as cobalt-zinc-cadmium resistance, mercuric resistance, arsenic resistance and copper tolerance, suggesting strain P40 has evolved with metal-resistant genes as a means of adaptation. With these heavy metal-resistance genes, *A. xenomutans* P40 may have a potential use in the bioremediation of heavy metal-contaminated environments. What's more, *A. xenomutans* P40 owned 136 genes related to "stress response". These genes would facilitate strain P40 surviving in the high-osmolality and cold environment. The high salt concentrations resulted in high osmotic stress. One effective strategy to maintain osmotic balance across a membrane is accumulating compatible solutes (Boscari et al., 2004). The presence of the choline dehydrogenase gene, high-affinity choline uptake protein gene and several glycine betaine transporters related genes indicated its strong tolerance to salts to synthesize and transport of betaine and choline. 21 cold/heat shock proteins could protect the cell from extreme temperatures (Weber and Marahiel, 2003). A number of genes that respond to oxidative stress were also identified such as genes coding for catalase, superoxide dismutase and so on.

Experiment showed that after 3 days culture of strain P40, 0.1% (w/v) hexadecane could be emulsification and degradation, thus conforming its good ability for alkane degradation. Genome analysis indicated that the genome of *A. xenomutans* P40 harbours three types of genes for alkane degrading, including three alkane hydroxylases (P40_00520, P40_03400, P40_21415), two P450 cytochrome (P40_09930, P40_11275) and one almA (P40_09820). Since the 16S rRNA gene shared 99.8%, the highest similarities with *A. dieselolei* B-5^T, and the average percentage of nucleotide sequence identity (ANI) showed 93.52% between *A. dieselolei* B5^T and strain P40, *A. dieselolei* B-5^T served as the closest phylogenetic neighbor for strain P40. Then comparative analysis was conducted between these two strains. Results

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