Complete genome sequence of a versatile hydrocarbon degrader, *Pseudomonas aeruginosa* DN1 isolated from petroleum-contaminated soil

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Abstract

Pseudomonas aeruginosa DN1 was isolated from a petroleum-contaminated soil from Changqing Oilfield with its capability to degrade high molecular weight polycyclic aromatic hydrocarbons (HMW PAHs) and crude oil. Herein, the whole genome sequence analysis of *P. aeruginosa* strain DN1 was reported, consisting of a size of 6,641,902bp chromosome assembled genome (67.09 mol% G+C content) and a 317,349bp plasmid assembled genome (57.01 mol% G+C content). According to the genome information, strain DN1 encodes various genes related to degradation of aliphatic hydrocarbons and aromatic compounds. In addition, DN1 contains gene clusters for biosynthesis and regulation of biosurfactant rhamnolipids. These genes may serve as a basis of further elucidation of the genetic background of this promising strain, and provide insights into investigating the metabolic and regulatory mechanisms of hydrocarbon biodegradation.

Key words

Complete genome sequence; *Pseudomonas aeruginosa* strain DN1; Hydrocarbon biodegradation; Synthesis of rhamnolipids

1. Introduction

Hydrocarbon pollutions have significant environmental consequences and present substantial hazards to human health and destroy the ecological balance, which may take years or even decades to recover. Numerous remediation technologies have been developed for the removal of hydrocarbon contamination, including physico-chemical and biological methods. Among these, bioremediation is one of effective clean-up strategies implemental for the restoration of hydrocarbon polluted environment due to its cost effectiveness, less stress to environment and complete recovery of contaminated sites (Kanaly and Harayama, 2010; Hu et al., 2013). Bacterial strains able to degrade hydrocarbons are known to be ubiquitous in nature. However, bioremediation efficiency can be limited by the bioavailability of hydrocarbons and survival of bacteria under adverse crude oil contaminated environment (Nikolopoulou and Pasadakis, 2013; Trelu et al., 2016). The isolation of indigenous bacteria is often beneficial since they can produce oil dispense compounds like biosurfactants that could accelerate hydrocarbon degradation (Ayed et al., 2015).

A number of microorganisms have potential on bioremediation of hydrocarbon contaminated environment (Lazar et al., 1995; Seo et al., 2009). Among these, *Pseudomonas aeruginosa* is well-known rhamnolipid producer, it is also considered to be ubiquitous hydrocarbon-degrader (Sajna et al., 2015). The type strain, DN1 was isolated from petroleum-contaminated soil in Shaanxi, China, and is already known to degrade various hydrocarbons as the sole carbon. Additionally, the strain DN1 had the ability of producing biosurfactant rhamnolipids in hydrocarbons rich culture medium (Ma et al., 2016). A phylogenetic tree, constructed by MEGA software (version 6.0) and neighbor-joining method, showed a distinct branch of DN1 with various *P. aeruginosa* strains, and strain DN1 was identified as the closest evolutionary relative of *P. aeruginosa* PAO 1 (Fig. 1). In order to gain insights into the genetic bases in its regulatory mechanisms for the further bioremediation study of hydrocarbon contaminants, we sequenced the whole genome of strain DN1, which was presented in this paper.

2. Data description

The whole genome sequencing of strain DN1 was performed using a combined strategy of PacBio RSII sequencing and Illumina Miseq paired-end sequencing platform with long-insert (10 kb) and short-insert (400bp) libraries, respectively. A total of 277038 reads were produced by the PacBio RS II sequencing. The quality of these reads was assessed by the FastQC tool, and the clean reads was *de novo* assembled by Celera Assembler. Gene prediction was carried out with the Glimmer 3.0 (Delcher et al., 1999) and gene annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline. The complete strain DN1 genome was composed of a circular 6,641,902bp chromosome and a circular 317.349bp plasmid. The chromosome harbored 6,215 open reading frames (ORFs), 64 tRNAs and 16 rRNAs with a GC content of 67.09%. The plasmid consisted of 469 open reading frames (ORF) with a GC content of 57.01%. Amongst the identified CDSs, 6185 were in the chromosome, and 264 were in the plasmid, respectively (Table1). A total of 5451 of the identified genes were classified to functional categories based on clusters of orthologous genes (COG) designation(Powell et al., 2014): 716 genes for general function prediction only, 549 genes for amino acid transport and metabolism, 495 genes for transcription, 353 genes for inorganic ion transport and metabolism, 327 genes for energy production and conversion, 326 genes for signal transduction mechanisms, 252 genes for cell wall/membrane/envelope biogenesis, 245 genes for lipid transport and metabolism, 226 genes for carbohydrate transport and metabolism, 198 genes for secondary metabolites biosynthesis, transport and catabolism, 196 genes for posttranslational modification and protein turnover and chaperones, 194 genes for translation and ribosomal structure and biogenesis, 185 genes for coenzyme transport and metabolism, 173 genes for replication and recombination and repair, 165 genes for intracellular trafficking and secretion and vesicular transport, 149 genes for cell motility, 98 genes for nucleotide transport and metabolism, 70 genes for defense mechanisms, 33 genes for cell cycle control and cell division and chromosome partitioning, 3 genes for chromatin structure and dynamics, 2 genes for RNA

processing and modification, and 496 genes for function unknown (Fig. 2).

Experimental findings from different research reports revealed that action of hydrocarbon utilizing bacteria on degradation and mineralization depended on the different catabolic genes and enzymes involved in a variety of known bacterial hydrocarbon degradation pathways and expression (Roy, 2013; Roy et al., 2014). For the metabolism of aromatic compounds, two metabolic pathways were well known, one leading to the formation of protocatechuic acid via o-phthalic acid and the other leading to catechol formation via salicylic acid. Subsequently, protocatechuic acid and catechol are degraded by ortho-cleavage and meta-cleavage pathways, respectively (Mishra et al., 2014). Across the genome of the strain DN1, plenty of genes and gene clusters could contribute to the degradation of aromatic compounds, such as *catA* (catechol 1, 2 dioxygenase, orf02054), *pcaG* (protocatechuate 3,4-dioxygenase subunit beta, orf00224) and *pcaH* (protocatechuate 3,4-dioxygenase alpha subunit, orf00226) (Mishra et al., 2014), homogentisate 1,2-dioxygenase (*hmgA*, orf01281), 2,4'-dihydroxyacetophenone dioxygenase (*dad*, orf04509), homoprotocatechuate 2,3-dioxygenase (*hpcB*, orf04547), benzoate/toluate 1,2-dioxygenase (orf01400, orf02068, orf02070, orf02071), gentisate 1,2-dioxygenase (orf01281, orf02001, orf06111), 4-hydroxyphenylpyruvate dioxygenase (orf06272), 4-hydroxyphenylacetate 3-monooxygenase (*hpaA*, *hpaC*, orf04505, orf04506), 4-hydroxybenzoate 3-monooxygenase (*pobA*, orf00357) (Gibson and Parales, 2000; Cao et al., 2015; Das et al., 2015). Moreover, many other genes were predicted to encode other enzymes involving in degradation of alkane and alkene, such as alkane 1-monooxygenase (*alkB1*, *alkB2*, orf02163, orf05286), polyhydroxyalkanoate synthesis protein (*phaF*, orf07819), 2-oxo-hepta-3-ene-1,7-dioic acid hydratase (*hpcG*, orf04550), 2,4-dihydroxyhept-2-ene-1,7-dioic acid aldolase (*hpaI*, orf04551), 2-nitropropane dioxygenase (orf06032) (Smits et al., 2002; Beilen et al., 2003; Hamme et al., 2003; Beilen and Funhoff, 2007). Notably, the key genes involved in degradation of alkane, alkene and aromatic compounds were identified in genome of strain DN1 with comparative genomic analysis of other bacterial genome from NCBI

database (Table 2). These results provide clues to certify that strain DN1 is a versatile hydrocarbon degrader. Moreover, strain DN1 genome encodes many genes involving in biosurfactant rhamnolipid synthesis, including rhamnosyltransferase (RhlA), glycosyl transferases (RhlB), L-rhamnosyl transferase (RhlC), dTDP-D-glucose 4,6-dehydratase (RmlB), dTDP-4-dehydrorhamnose reductase (RmlD), glucose-1-phosphate thymidyltransferase (RmlA), dTDP-4-dehydrorhamnose 3,5-epimerase (RmlC) and phosphomannomutase (AlgC). Additionally, there are many gene regulatory factors related to rhamnolipid production, such as RsaL, RpoN, RpoS, PhoB, DksA, MvfR, PtxR and AlgR (Reis et al., 2011; Dobler et al., 2015; Lovaglio et al., 2015).

The genome information of strain DN1 provides insights into investigating the metabolic and regulatory mechanisms of hydrocarbon biodegradation. The complete genome chromosome and plasmid sequences have been deposited in the DDBJ/EMBL/GenBank database with accession number CP017099 and CP018048, respectively.

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Table 1 General genome features of *P. aeruginosa* DN1

Feature	Chromosome	Plasmid
Length (bp)	6,641,902	317,349
G+C content (%)	67.09	57.01
Protein coding genes (CDSs)	6185	264
rRNA number	12	0
tRNA number	64	0
ncRNA	78	0
GenBank accession	CP017099	CP018048

Table 2 Comparison of gene clusters related to degradation of chain hydrocarbon and aromatic compounds in *P. aeruginosa* DN1 and other *P. aeruginosa* strains retrieved from <http://www.pseudomonas.com/>

Strain	dioxygenase					monooxygenase				
	<i>pcaH</i>	<i>pcaG</i>	<i>catA</i>	<i>hmgA</i>	<i>dad</i>	<i>pobA</i>	<i>hpaA</i>	<i>hpaC</i>	<i>alkB1</i>	<i>alkB2</i>
<i>P. aeruginosa</i> PAO1	●	●	●	●		○	○	○	○	○
<i>P. aeruginosa</i> PA7	●	●	●	●		○	○	○		
<i>P. aeruginosa</i> DN1	●	●	●	●	●	○	○	○	○	○
<i>P. aeruginosa</i> CI27	●	●		●	●	○	○	○	○	○
<i>P. aeruginosa</i> CIG1	●	●		●	●	○	○	○	○	○
<i>P. aeruginosa</i> PAO581	●	●	●	●		○	○	○	○	○

Fig. 1 Phylogenetic tree of *Pseudomonas aeruginosa* DN1 (The tree was constructed using software MEGA 6.0 by neighbour-joining method based on 16S rDNA gene sequences with 1000 replications in bootstrap test)

Fig. 2 COG function classification of *P. aeruginosa* DN1