Identification and genome analysis of *Deinococcus actinosclerus* SJTR1, a novel 17 β -estradiol degradation bacterium

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Abstract

Biodegradation with microorganisms is considered as an efficient strategy to remove the environmental pollutants. In this work, *Deinococcus actinosclerus* SJTR1 isolated from the wastewater was confirmed with great degradation capability to 17 β -estradiol, one typical estrogen chemical. It could degrade nearly 90% of 17 β -estradiol (10 mg/L) in 5 days and transform it into estrone; its degradation kinetics fitted for the first-order kinetic equation. The whole genome sequence of *D. actinosclerus* SJTR1 was obtained and annotated, containing one chromosome (3,315,586 bp) and four plasmids (ranging from 17,267 bp to 460,244 bp). A total of 3913 CDSs and 73 RNA genes (including 12 rRNA genes, 50 tRNA genes, and 11 ncRNA genes) were identified in its whole genome sequence. On this basis, a series of potential genes involved in steroid metabolism and stress responses of *D. actinosclerus* SJTR1 were predicted. It is the first report of *Deinococcus* strain with the degradation capability to estrogens. This work could enrich the genome sources of the estrogen-degrading strains and promote the degradation mechanism study of 17 β -estradiol in bacteria.

Keywords Biodegradation · *Deinococcus actinosclerus* · 17 β -Estradiol · Whole genome sequence · Estrogen degradation

Introduction

Environmental estrogens (EEs) are one of the most important environmental pollutants as their long environment remains, adverse health effects, and low removal efficiency (Bulzomi et al. 2010). 17 β -Estradiol (E2) exerts the highest estrogenic activity among the typical natural estrogens. Biodegradation with microorganisms is considered as an efficient strategy to remove the EEs pollution and remediate the polluted environment. Series of bacteria with estrogen degradation capabilities have been isolated from various environmental niches, such as *Sphingomonas*, *Pseudomonas*, *Acinetobacter*, *Rhodococcus* and *Bacillus* (Ke et al. 2007; Li et al. 2018; Liang et al. 2012; Roh and Chu 2010; Yu et al. 2013, 2007). Their degradation efficiency to the estrogenic

chemicals varied a lot. For example, *Sphingomonas* sp. KC8 could degrade 3 mg/L of E2 completely in 5 days; while *Bacillus* spp. E2Y1 need 6 days to degrade 1 mg/L of E2 (Roh and Chu 2010). However, as only few estrogen-degrading strains have been sequenced, the degradation mechanism of estrogens in bacteria is still unclear (Zheng et al. 2016; Liang et al. 2012; Chen et al. 2017; Li et al. 2018).

In this study, one 17 β -estradiol-degrading bacteria strain SJTR1 was isolated from the wastewater collected in the wastewater treatment plant of Shanghai, China. The isolated bacterium is aerobic, Gram-negative, short-rod shaped, pink-red pigmented, whose optimum growth was 30 °C. Its genomic DNA was extracted with Bacterial Genomic DNA Extraction Kit (TIANGEN, Beijing, China), and the 16S rRNA gene was amplified using 16S rDNA Bacterial Identification PCR Kit (RR176, TaKaRa, Dalian, China). Its 16S rDNA fragment was sequenced with primers: 5'-GAGCGG A TAACAATTTTCACACAGG-3' and 5'-CGCCAGGGTTTT CCCAGTCACGAC-3', and the sequence of 16S rDNA was deposited into GeneBank (accession no. MH179322.1). Taxonomic analysis based on the 16S rDNA sequences revealed that strain SJTR1 was clustered with the members of the species *Deinococcus actinosclerus* and *Deinococcus arenae* (Fig. 1). The ANI analysis was performed for more detailed

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Fig. 1 The phylogenetic tree constructed on the basis of the 16S rDNA sequences. The phylogenetic tree was constructed with MEGA 7.0 software using the neighbor-joining method with Kimura two-parameter model. The bootstrap consensus tree was performed with 1000 replicates

phylogenetic classification after the whole genome sequencing (Figueras et al. 2014). On the basis of comparison with the 14 most closely related strains, strain SJTR1 was correlated with *D. actinosclerus* BM2 with the highest genomic similarity (95.66% similarity with 85.76 of the total genome alignment) (Table S1). The Tetra Correlation Search (TCS) of the closest sequenced bacteria in the JSpeciesWS database also ranked *D. actinosclerus* BM2 as the closest strain of strain SJTR1 with a Z-score of 0.99877 (Table S2). Taken together, strain SJTR1 was identified as a new member of *D. actinosclerus*.

Then, the degradation efficiency of *D. actinosclerus* SJTR1 to 17 β -estradiol was determined by detecting the residues of 17 β -estradiol in culture with high-performance liquid chromatography (HPLC). As shown in Fig. 2, *D. actinosclerus* SJTR1 could utilize 17 β -estradiol as its sole carbon source and exhibit significant degradation efficiency; 10 mg/L of 17 β -estradiol could be removed nearly 90% within 5 days. Estrone (E1) was the primary metabolite of 17 β -estradiol, whose concentration reached the peak at the 4th day and then decreased gradually to the final

Fig. 2 The degradation kinetics of 17 β -estradiol and cell growth of *D. actinosclerus* SJTR1. *D. actinosclerus* SJTR1 was inoculated in the minimal medium containing 10 mg/L of E2 and cultured for 7 days. The cell growth (dashed line, filled circles), the residue of 17 β -estradiol in culture (solid line, filled circles) and the production of estrone in culture (solid line, filled squares) was monitored and plotted. Control represented the non-inoculation treatment (solid line, filled triangles). The error bars stand for the errors of triplicate tests

concentration lower than 2 mg/L. Its degradation kinetics fitted for the first-order kinetic equation $C = 10.455e^{-0.29t}$ with $R^2 = 0.9159$, where C represented the E2 concentration in culture (mg/L) and t was the cultivation time (day). The half-life of biodegradation by *D. actinosclerus* SJTR1 to 17 β -estradiol (10 mg/mL) was about 3 days. Comparing with other reported strains like *Sphingomonas* sp. KC8 and *Bacillus* spp. E2Y1, *D. actinosclerus* SJTR1 showed higher degradation efficiency and shorter degradation period (Roh and Chu 2010). More important, although the members of *Deinococcus* genus are known as their great resistance to environmental hazards, their degradation capabilities to environmental pollutants are rarely concerned. *D. actinosclerus* SJTR1 is the first *Deinococcus* strain reported with the degradation capability to estrogenic chemicals (Fig. 3).

To gain insights on the degradation mechanism of *D. actinosclerus* SJTR1 to estrogenic chemicals, its whole genome was sequenced. The genomic DNA of *D. actinosclerus* SJTR1 was extracted and purified using QIAamp DNA Mini Kit (Qiagen, CA, USA). The quantity and quality of the extracted genomic DNA were evaluated on the Agilent 2100 Bioanalyzer (Agilent, US). The genome DNA was used to construct two libraries. One was a 10 kb insert SMRT-bell library, which was sequenced on the single molecule real-time (SMRT) DNA sequencing platform by Pacific Biosciences (PacBio) RS II sequencer (Pacific Biosciences, CA, USA) (Eid et al. 2009). Another one was a 400 bp insert library, which was sequenced with Illumina

Miseq platform (Glenn 2011). Then specific PCR amplification and Sanger sequencing were used to close the gaps.

The whole genome sequence of *D. actinosclerus* SJTR1 was revealed using two SMRT cells with a single 180 min movie (Pacific Biosciences, US), and the raw data was obtained as 299,742 reads for 2,415,924,050 bp. The data acquired by Illumina Miseq platform with paired-end sequencing mode (2×250 bp) was contained 17,722,564 reads for 4,396,879,518 bp. The sequenced reads of PacBio RS II was de novo assembled using the Celera® Assembler and PBjelly software (Denisov et al. 2008); the Illumina Miseq data were corrected with Kmer and assembled with Newbler (Fletez-Brant et al. 2013; Margulies et al. 2005). The genome sequence was annotated with RAST (Aziz et al. 2008). The coding sequences (CDSs) were predicted using Glimmer 3.0 (Delcher et al. 1999), and gene functions were annotated with NCBI-NR (Benson et al. 2003), eggno3 (Powell et al. 2014), Swiss-port (Boutet et al. 2016), and KEGG databases (Kanehisa et al. 2008).

The complete genome of *D. actinosclerus* SJTR1 is composed of one chromosome of 3,315,586 bp with an average GC content of 70.72% and four plasmids [base pairs and GC contents: 30,970 bp (62.71%), 187,951 bp (67.72%), 460,244 bp (71.69%), 17,267 bp (62.14%)] (Table 1). Among the 3892 genes in the genome, 3303 genes were predicted to be the coding sequences (CDSs); in the plasmids, the number of coding genes varied from 27 to 375. In addition, the genome encodes 49 tRNA genes, 11 ncRNA genes and nine rRNA genes (two 5S–16S–23S rRNA operons and

Fig. 3 Genome map of *D. actinosclerus* SJTR1. The chromosome and the four plasmids of *D. actinosclerus* SJTR1 were shown. From the inside to outside was the sequence scale mark, GC skew (green

and violet), GC content (black), COG for each CDS (colored by COG categories) and the positions of CDS, rRNAs and tRNAs on the genome (blue)

Table 1 General features of *D. actinosclerus* SJTR1 genome

Genome features	Chromosome	Plasmid 1	Plasmid 2	Plasmid 3	Plasmid 4
Genome size (bp)	3,315,586	30,970	187,951	460,244	17,267
GC content (%)	70.72	62.71	67.72	71.69	62.14
Total genes	3892	61	227	461	38
Protein-coding genes	3303	45	163	375	27
rRNA genes	9	0	0	3	0
(5S, 16S, 23S)	(3: 3: 3)			(1: 1: 1)	
tRNA genes	49	0	0	1	0
Other ncRNA genes	11	0	0	0	0

three distributed rRNA); the plasmid 3 also contain distributed three rRNA genes and one tRNA gene (Table 1).

Further analysis showed that the majority of predicted proteins could be classified into 25 COG categories. The five most abundant subsystems are related to amino acid metabolism ($n=266$), carbohydrates metabolism ($n=171$), translation, ribosome structure and biogenesis ($n=159$), transcription ($n=152$), and energy production and conversion ($n=134$). The general genome features of six closest *Deinococcus* strains were compared and results indicated that the genome size of the seven strains ranged from 2.6 M bp to 3.9 M bp with high GC contents; some of them also contained plasmids (Table 2). *Deinococcus* genus is famous for its strong ability to survive in the extreme environments; for example, *D. actinosclerus* BM2, *Deinococcus swuensis* DY59, and *Deinococcus puniceus* DY1 was found with great tolerance to the Gamma radiation (Kim et al. 2016; Lee et al. 2013, 2015). These was mainly due to their strong DNA repair system genes and multiple stress response genes (Agapov and Kulbachinskiy 2015; Gerber et al. 2015; Krisko and Radman 2013; Slade and Radman 2011). Genome mining on the genome of *D. actinosclerus* SJTR1 showed that there were 141 genes related to stress responses, in which 63 genes were participated in the DNA repair system and 44

genes were involved in the oxidative responses (Table S3). These findings revealed the genome basis of *D. actinosclerus* SJTR1 for its diverse catabolic capacities and unique abilities to adapt and survive in different stress and adverse environments.

Although the genetic basis for estrogens biodegradation in bacteria is still not very clear, the transformation from 17 β -estradiol to estrone is considered as the first and key step for the degradation of this chemical (Chen et al. 2017; Yu et al. 2013). In human, multiple 17 β -hydroxysteroid dehydrogenases (17 β -HSDs) have been confirmed to catalyze the oxidation/reduction reaction of 17 β -estradiol and estrone (Labrie et al. 1997). In bacteria, several enzymes belonging to the short-chain dehydrogenase/reductase (SDR) superfamily were also identified with similar function (Chen et al. 2017; Ye et al. 2017; Yu et al. 2015). In *D. actinosclerus* SJTR1, genome analysis showed that there were 18 genes encoding SDRs, probably contributing to the transformation from 17 β -estradiol to estrone (Table S4). Five proteins showed the high homology to the characterized 17 β -HSDs of *Sphingomonas* strain KC8 [WP_062158034.1 (31%), WP_062158570.1 (30%), WP_062157977.1 (30%), WP_062159023.1 (30%) and WP_062156992 (27%)]. Multiple sequences alignment and secondary structure analysis

Table 2 General genome features of seven *D. actinosclerus* strains

Genome features	<i>Deinococcus actinosclerus</i> SJTR1	<i>Deinococcus actinosclerus</i> BM2	<i>Deinococcus swuensis</i> DY59	<i>Deinococcus puniceus</i> DY1	<i>Deinococcus peraridilitoris</i> DSM 19,664	<i>Deinococcus maricopensis</i> DSM 21,211	<i>Deinococcus wulumuqiensis</i> NEB 479
Genome size (bp)	3,315,586	3,264,334	3,531,443	2,971,983	3,881,839	3,498,530	2,658,929
Plasmids	4	0	0	0	2	0	6
GC content (%)	70.72	70.60	67.41	62.65	63.98	69.83	66.82
Protein-coding genes	3913	3255	2904	2617	3653	3275	3384
RNA genes	73	59	55	57	72	67	59
rRNA genes	12	9	7	9	12	12	9
(5S, 16S, 23S)	(4, 4, 4)	(3, 3, 3)	(2, 3, 2)	(3, 3, 3)	(4, 4, 4)	(4, 4, 4)	(3, 3, 3)
tRNA genes	50	49	47	47	58	53	47
Other ncRNA genes	11	1	1	1	2	2	3

Fig. 4 Multiple Sequences alignment (MSA) of 17 β -HSDs. The sequences of 17 β -HSDs from *Sphingomonas* strain KC8 (Sequence ID: ARS27503.1), *Homo sapiens* (Sequence ID: NP_568742.1), and the sequences of five SDRs from *D. actinoscleris* SJTR1 (WP_062158034.1, WP_062158570.1, WP_062157977.1, WP_062159023.1 and WP_062156992) were aligned with MAFFT. The conserved amino acids were indicated in color. The secondary structures of seven α -helices and seven β -strands were marked

showed that several high conserved amino acids and motifs were all observed in the five potential SDRs and the two reported 17 β -HSDs (Fig. 4). In addition, further complex metabolic pathway is also needed for the further transformation of produced estrone into carbon dioxide and water, that means, a series of enzymes should work collaboratively to open the aromatic rings and break the side chains (Chen et al. 2017; Lee and Liu 2002; Nakai et al. 2010). At least 13 dioxygenase-encoding genes and 119 different dehydrogenase-encoding genes were also found in the genome of *D. actinosclerus* SJTR1, which were speculated to participate in the further degradation (Table S4).

In conclusion, the characterization of *D. actinosclerus* strain SJTR1 and the analysis of its whole genome sequence can enrich the genome database of estrogens-degrading microorganisms, shed a light on the biodegradation mechanism study of estrogens, and contribute to its future applications in environment remediation.

Nucleotide sequence and strain accession number

The complete genome sequence of *D. actinosclerus* SJTR1 has been deposited at GeneBank under the accession no. CP029774 (chromosome), CP029775 (plasmid 1), CP029776 (plasmid 2), CP029777 (plasmid 3) and CP029778 (plasmid 4). This strain has been deposited in China General Microbiological Culture Collection Center (CGMCC) with accession no. 15630.

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Compliance with ethical standards

Conflict of interest The authors declared no conflict of interest.

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