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Isolation and genome sequence analysis of a bacterium degrading dexamethasone.

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Abstract

In this study, we reported the genome sequence of *Burkholderia* sp.CQ001, a Dexamethasone (DXM) degrading bacterium isolated from hospital wastewater and identified by morphological analysis, staining and 16S rRNA sequencing. The degradation rates of dexamethasone sodium phosphate and dexamethasone were 84.8% and 77.11% respectively. And degradation peak appeared at 24 h during agitation culture at 37°C in medium with an initial pH value of 7.5. Genome sequencing was performed using Illumina Hiseq4000 high-throughput sequencing platform. Genome sequencing results demonstrated that the bacteria had a total genome size of 7570308 bp, 66.9% G+C content. Metabolic related genes account for 80.3%. Metabolism-associated genes covered 116 metabolic pathways, including metabolic pathways of microorganisms in different environments and decomposition pathways of secondary metabolites. One of the eight key genes annotated to the metabolic pathway of steroid compounds. This is the first report on *Burkholderia* sp.CQ001 showing characteristics of degrading dexamethasone. Our findings may provide insights on dexamethasone degradation mechanisms, and facilitate the establishment of bioremediation engineered bacteria to eliminate the dexamethasone pollution.

Keywords: Dexamethasone, Steroid hormones, Degradation, *Burkholderia*, Genome sequence.

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Introduction

Dexamethasone (DXM), synthetic long-acting glucocorticoids, has been extensively used in the prevention and treatment of various human and poultry diseases in the past decades [1-3]. Dexamethasone Sodium Phosphate (DSP) is the most commonly used formulation of DXM in clinical practice due to its water solubility and absorbability [4]. However, long-term or high-dose use of DXM frequently leads to severe adverse events including Cushing's syndrome, cardiovascular disease, osteoporosis, or aseptic necrosis of the femoral head [5-7]. Previous studies have reported that DXM residues may cause environmental pollution through a variety of ways including industrial, hospital, or domestic wastewater, or even drinking water [8-11]. Therefore, studies on dexamethasone degradation mechanisms are important in establishment of bioremediation engineered bacteria to eliminate the dexamethasone pollution.

Burkholderia is a gram-negative bacterium distributed widely in water, soil, plants and human body [12,13]. It has a unique metabolic potential that can grow and reproduce with more than 200 kinds of organic compounds as a carbon source [13]. The genome sequences of some strains of the *Burkholderia* genus have been disclosed, and the whole genomic information of *B. vietnamiensis* strain G4 and *B. xenovorans* LB400 with the property of degradation have been reported [14]. However, the degrading effects on steroids and their related genes by these strains have not been investigated.

In this study, a strain using dexamethasone sodium phosphate as the sole carbon source and energy sources were successful obtained and identified as *Burkholderia* and was named as *Burkholderia* sp.CQ001. Based on the genome sequence and bioinformatics analysis, we found that *Burkholderia* sp.CQ001 had genes and metabolic pathways that can potentially degrade dexamethasone steroids with high efficiency.

Experiments

Isolation and identification of bacteria

One bacterial strain was isolated from the wastewater of a hospital in Chongqing, China (29° 33'N, 106° 28'E) using the methods reported by Wang et al. [15]. The solid phase

extraction-High Performance Liquid Chromatography (HPLC) method was used to detect the degradation effects of the bacteria on DXM and DSP (500 μg/ml), and the effects of initial medium pH and temperature on the degradation.

Finally, the morphological features of bacteria were observed by electron microscopy and scanning electron microscopy after Gram-staining. The main biochemical reactions were detected by conventional methods. The taxonomic status of the bacteria was identified by 16s rRNA sequencing.

Homology and phylogenetic analysis

Genomic DNA was extracted using DNA preparation kit according to the manufacturer's instructions (Takara, Japan). The *16s rRNA* gene sequences of *Burkholderia* sp.CQ001 were amplified by PCR with universal primers of *27F* and *1492R*. The genomic sequencing for amplification products was conducted using sequencer of ABI3730XL at Majorbio Bio-pharm Technology Company (Shanghai, China), and sequence analysis was carried out in the NCBI database. Meanwhile, The *16s rRNA* full sequences of 16 bacterial strains with high similarity to the sequenced strains were downloaded from NCBI GenBank, and the multiple sequence comparison was conducted using CLUSTAL W [16]. Ultimately, comparative results were analysed by MEGA 6.0 [17] using neighbor-joining method (bootstrap=1000) to build the phylogenetic tree [18].

Genome sequencing and assembly

Genome sequencing was performed at Majorbio Bio-Pharm Technology company (Shanghai, China) using Illumina Hiseq4000 high-throughput sequencing platform by building a ~500 bp Paired-End library. After handling of the raw data, the optimized sequences were spliced using SOAPdenovo software (version 2.04) [19]. The partial filling and base correction were carried out for the optimal assembly sequences obtained through the above-mentioned procedures by GapCloser software (version 1.12). Ultimately, the filled and corrected sequences were matched to the measured sequences (reads), and assembled results were evaluated by statistics analysis for the depth of coverage between the content of G+C and reads. A total of 1,390,912 of original sequences were identified, and 12,891,489 high-quality sequences were obtained after quality cutting.

Genome annotation

The *rRNAs* and *tRNAs* genes included in the genome were predicted using RNAmmer (version 1.2) [20] and tRNAscan-SE (version 1.3.1) [21] software, respectively. The protein-coding sequence was predicted using Glimmer software (version 3.02) [22]. To obtain functional annotation information of COG [23], GO, KEGG [24] and other relevant database, a blast alignment (BLAST 2.2.28+) was conducted between the predicted sequences and Nr, genes, string and GO database. Blast alignment information was adopted only when E values ≤ 10^{-5}. The relevant functional genes that may be involved in the degradation of DXM were assessed.

Comparative genomics analysis

Next, we analysed the basic genomic features of obtained sequences including gene sequence length (bp), G+C percentage, number of predicted genes, and number of encoding RNA *(tRNA* and *rRNA*). Then, these features of obtained sequences were compared to the sequences of 6 representative strains of *Burkholderia* genus selected from NCBI database.

Results and Discussion

Biological characteristics of bacteria

The isolated bacteria with potential of degrading DXM/DSP were gram-negative bacillus with straight or slightly curved cylinder shape, and 6-15 μm × 4-^{15} μm in length and diameter. On LB agar plates, the bacteria formed a milky white opaque colony with medium size (d=1.5-2.0 mm), round, smooth and moist surface, regular edge, and easy to be stirred up (Figure 1). The catalase and oxidase tests showed positive results. The bacteria were initially identified by *16s rRNA* sequencing, which belonged to the genus *Burkholderia* and were named as *Burkholderia* sp.CQ001. A *16srRNA*-based phylogenetic tree was constructed (Figure 2), which revealed *Burkholderia* sp.CQ001 having closest kinship with *Burkholderia* contaminants J2956 in evolution.

These bacteria can degrade DSP into DXM and then degrade the latter into other small molecules, with the degradation rates of 84.8% and 77.11%, respectively. And degradation peak appeared at 24 h during agitation culture at 37°C in medium with an initial pH value of 7.5.

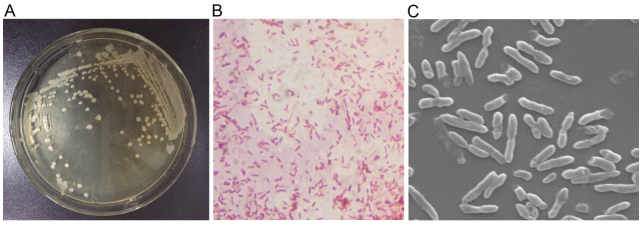

Figure 1. *Morphological characteristics of Burkholderia sp.CQ001. A: Morphology of colony on LB agar plate; B: Gram staining as shown under Electron microscope (1000X); C: Scanning electron microscope (4000X).*

The basic characteristics of the genome

Burkholderia sp.CQ001 had a total genome size of 7570308 bp, and contained 66.9% G+C. The 8,705 predicted genes included 8,632 protein-coding genes and 73 RNA coding genes (15 *rRNA* and 58 *tRNA*). The statistical information of gene prediction results was demonstrated in Table 1.

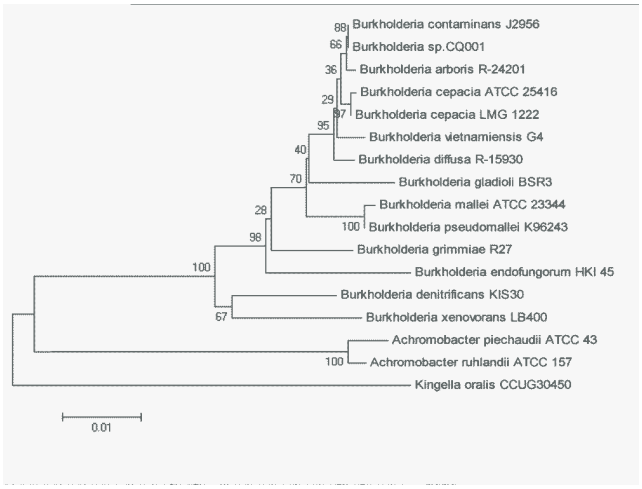

Figure 2. *Phylogenetic tree analysis of Burkholderia sp.CQ001, with bootstrap=1000.*

Gene annotation and function classification

COG function classification: The alignment results compared with COG sequences were shown in Figure 3A, about 70.1% genes were annotated. There were 408 genes for energy production and Conversion (C), 395 for carbohydrate transport and metabolism (G), 260 for secondary metabolism biosynthesis, transportation and catabolism (Q), 135 for intracellular trafficking, secretion and vesicular transportation (U). These genes may be closely related to the degradation of dexamethasone of the bacteria. Depending on COG classification, the functional gene of *Burkholderia* sp.CQ001 was classified into four categories (Table 2). Therefore, we speculate that the bacteria have a unique metabolism potential that can grow and reproduce with DSP as the sole carbon source and survive in the extreme environment just like hospital wastewater.

KEGG metabolic pathway the specific genes involved biological pathways can be achieved through KEGG analysis (Figure 3B). About 80.3% genes were metabolism-associated and they covered 116 metabolic pathways, including the metabolic pathways of microorganisms in different environments and decomposition pathways of secondary metabolites. More importantly, there were also 165 genes of degradation and metabolism for xenobiotics (A: Metabolism/ Xenobiotics biodegration and metabolism). Among them, 8 pathways involved in the metabolism of steroid compounds can encode 7 key enzymes (Table 3). According to the steroid degradation pathway (http://www.genome.jp/kegg/ pathway.html), the metabolic pathways of steroid compounds in *Burkholderia* sp.CQ001 KEGG pathway are as the follows: 3β-HSD continuously catalyses the 3β-OH steroid compounds dehydrogenation and isomerism reaction, facilitating the first step in the ring opening reaction of the steroid nucleus [25,26]. The interaction of KstD and KSH induces the cleavage of the B ring of the steroid nucleus [27]. KSH is composed of KshA and KshB. The Hsa family proteins catalyze the cleavage of the steroid nucleus into ATP and small molecule organic salts and coenzymes [28]. The presence of these genes and pathways provides a basis for the degradation of steroid hormones by *Burkholderia* sp.CQ001.

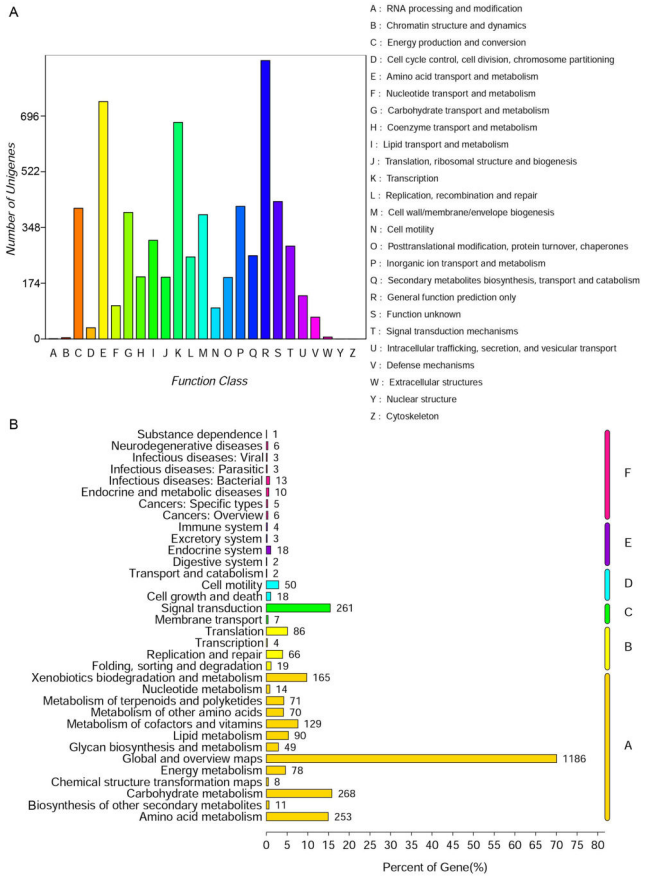

Figure 3. *Gene annotation function classification chart of Burkholderia sp.CQ001. A: Function classification chart of COG. The functional gene of Burkholderia sp.CQ001 was classified into four categories: energy production and Conversion (C), carbohydrate transport and metabolism (G), secondary metabolism biosynthesis, transportation and catabolism (Q), intracellular trafficking, secretion, and vesicular transportation (U); B: Function classification chart of KEGG secondary metabolites. About 80.3% genes were metabolism-associated and with metabolism covered 116 metabolic pathways, including the metabolic pathways.*

Table 1. *General features of Burkholderia sp. CQ001 genome.*

Features	Number
Gene number	8705
Gene total length	7570308 bp
Gene average length	869 bp
Gene density	0.946 genes per kb
GC content in gene region (%)	66.9
Gene/Geonme (%)	82.3

Comparative genomics information

Burkholderia genus, with abundant genetic diversity, has more than 40 strains including bacteria that are pathogenic to human,

animal and plant and bacteria can degrade organic matter. The basic genomic characteristics of *Burkholderia* sp.CQ001 were compared to the most representative strains selected from each of the *Burkholderia* strains (Table 4). Chain et al. [14] revealed that *Burkholderia* xenovorans LB400, a non-pathogenic bacterium isolated from the sewage, had enriched degradation pathways for aromatic compounds, including 11 major trichloromethyl aromatic compounds and more than 20 minor aromatic compounds, such as biphenyl, diaminophenol, and trichloro-cresol. Furthermore, the preliminary comparison demonstrated that *Burkholderia* sp.CQ001 had the closest number of predicted genes with *Burkholderia xenovorans* LB400, and a wealth of degradation pathways of aromatic compounds were also found in KEGG pathways analysis. Thus, we speculate that these two strains share a high degree of similarity in the gene structures and functions. However, the degradation of steroidal compound by *Burkholderia xenovorans* LB400 and by other *Burkholderia* strains has not been reported. More degradation related genes in *Burkholderia* sp.CQ001 may be disclosed in further comparative analysis of these two bacteria.

Table 2. *COG distribution of Burkholderia sp.CQ001.*

Category	Group	Function	Number of genes
Information storage and processing	A	RNA processing and modification	1
	B	Chromatin structure and dynamics	4
	J	Translation, ribosomal structure and biogenesis	193
	K	Transcription	676
	L	Replication, recombination and repair	253
Cellular processes and signaling	D	Cell cycle control, cell division, chromosome partitioning	35
	M	Cell wall/membrane/envelope biogenesis	388
	N	Cell motility	97
	O	Posttranslational modification, protein turnover, chaperones	192
	T	Signal transduction	290
	U	Intracellular trafficking, secretion and vesicular transport	135
	V	Defense mechanisms	68
	W	Extracellular structures	6
Metabolism	C	Energy production and conversion	408
	E	Amino acid transport and metabolism	741
	F	Nucleotide transport and metabolism	104
	G	Carbohydrate transport and metabolism	395
	H	Coenzyme transport and metabolism	194
	I	Lipid transport and metabolism	308
	P	Inorganic ion transport and metabolism	414
	Q	Secondary metabolism biosynthesis, transport and catabolism	260
Poorly characterized	R	General function prediction only	896
	S	Function unknown	429
Total			6487

Table 3. *Steroid compounds-degrading enzymes in Burkholderia sp.CQ001 genome.*

Degrading enzymes	Definitions	ORF	Orthologous genes
HsaA	3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione monooxygenase (EC:1.14.14.12)	orf00263_1	*K16047*

		orf00460_1	*K16047*
HsaC	3,4-dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione 4,5-dioxygenase (EC:1.13.11.25)	orf00274_1	*K16049*
HsaD	4,5:9,10-diseco-3-hydroxy-5,9,17-trioxoandrosta-1(10),2-diene-4-oate hydrolase (EC:3.7.1.17)	orf00306_1	*K16050*
KstD	3-oxosteroid 1-dehydrogenase (EC:1.3.99.4)	orf00265_1	*K05898*
KshA	3-ketosteroid-9-alpha-hydroxylase oxygenase subunitA (EC:1.14.13.142)	orf00291_1	*K15982*
KshB	3-ketosteroid-9-alpha-hydroxylase reductase subunitB (EC:1.14.13.142)	orf00267_1	*K15983*
3β-HSD	3(or 17)beta-hydroxysteroid dehydrogenase (EC:1.1.1.51)	orf00350_1	*K05296*

Table 4. *Comparison of basic genome characteristics between Burkholderia sp.CQ001 and other strains of Burkholderia.*

Genome	**Length (bp)**	**G+C (%)**	**Genes**	***rRNA* genes**	***tRNA* genes**	**Isolated**	**Signal P (%)**	**NCBI accession**
Burkholderia xenovorans LB400	9702951	62.6334	8596	18	65	Polluted water	9.52	GCF_000756045.1_ASM75604v1
Burkholderia vietnamiensis G4	8391070	65.7381	7592	18	68	Polluted water	9.32	GCF_000016205.1_ASM1620v1
Burkholderia mallei ATCC 23344	6935527	68.4885	5506	10	56	Human	7.39	GCF_000011705.1_ASM1170v1
Burkholderia cenocepacia J2315	8055782	66.8993	7273	18	73	Human	11.48	GCF_000009485.1_ASM948v1
Burkholderia pseudomallei K96243	7247547	68.0587	5935	12	61	Human	10.34	GCF_000011545.1_ASM1154v1
Burkholderia gladioli BSR3	9052299	67.3975	7708	15	69	Plants	9.98	GCF_000194745.1_ASM19474v1
Burkholderia sp. CQ001	9192367	66.015	8705	15	58	Polluted water	7.42	SRP073478

Conclusions

At present, the studies on the degradation mechanism of steroids are mainly involved in *Streptococcus* and *Mycobacterium* [29-31]. Among the *Burkholderia*, No related gene or metabolic pathway of the degradation of dexamethasone has been found. Here we report *Burkholderia* sp.CQ001, a Dexamethasone (DXM) degrading bacterium successfully isolated from hospital wastewater. The degradation rates of dexamethasone sodium phosphate and dexamethasone were 84.8% and 77.11% respectively. Our study gives a description of the genome sequence of *Burkholderia* sp.CQ001 and a preliminary analysis of related functional genes and metabolic pathways. More importantly, the findings provide reference for further studies on dexmethasone degradation mechanisms and establishment of bioremediation engineered bacteria to eliminate the dexamethasone pollution.

Registration number of gene sequences

The genome sequences of *Burkholderia* sp.CQ001 were stored in the DDBJ/EMBL/GenBank database under accession number PRJNA339502.

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Conflict of Interest

The authors declare no conflict of interest.

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