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Draft genome sequence of highly efficient arsenite-oxidizing bacterium *Methylobacterium brachythecii* C25, isolated from arsenic-contaminated soil

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비소오염 토양 유래 세균 *Methylobacterium brachythecii* strain C25의 유전체

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Here, we report the features and draft genome sequence of *Methylobacterium brachythecii* C25, isolated from the agricultural soil contaminated with arsenic (As) in Republic of Korea. This strain is able to reduce the toxicity by oxidizing arsenite (As [III]) to arsenate (As [V]) while having resistance to high concentration of As (III). The genome consists of 5,191,050 bp chromosome with a GC content 66.03%, which contains 4,975 coding DNA sequences (CDSs), 49 tRNA genes, 3 rRNA genes, 4 non-coding RNA genes, and 75 pseudo genes. The genome also contains two types of As (III) oxidase (Aio, arsenite oxidase gene) and several stress resistance genes to As (III). Taken together, strain C25 is not only able oxidize As (III) to As (V) but also has resistance to As-contaminated environmental stress.

Keywords: *Methylobacterium brachythecii*, arsenite, biological oxidation, draft genome

Arsenic (As) as a known human carcinogen is a toxic metalloid widely present in the environment (Santini *et al*., 2000; Butt *et al*., 2011; Xiao *et al*., 2021). As accumulation in soil and water is mainly due to the anthropogenic activities such as use of pesticide, combustion of fossil fuels, mining and/-or smelting, and wastewater irrigation (Das *et al.*, 2014). The predominant forms of As found in water or soil environments are inorganic As (III) (arsenite) and As (V) (arsenate) (Santini *et al*., 2000; Butt *et al*., 2011; Pous *et al.*, 2015). As (III) is reported to be more harmful to human health than As (V) because of its higher toxicity, solubility, and mobility (Xiao *et al*., 2021). Microbial oxidation of As (III), which is being cheap and self-regenerating catalyst, is recently attracting considerable attention (Shi *et al*., 2020). In spite of the fact that understanding the mechanism or metabolism of As (III) oxidation is very important, the genetic information of the newly isolated bacterial strain, which may even suggest a novel metabolic pathway, is not yet understood (Xiao *et al*., 2021).

The As (III)-oxidizing bacterium *Methylobacterium brachythecii* C25 (KACC 19855) was isolated from the As-contaminated agricultural soil. Contaminated samples were collected at the site of an agricultural soil in the South

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Chungcheong province (South Korea). The enrichment cultures were conducted aerobically at 30°C and 150 rpm for 2 weeks, using mineral salt medium (Kumar and Maitra, 2016) with 1 mM of As (III). The enrichment-cultured samples were diluted in phosphate-buffered saline (PBS) (pH = 7.4). The diluted samples were then spread on R2A agar plates (BD Difco) and incubated at 25°C and 150 rpm for 2 weeks to obtain a single colony. To determine the oxidation capability of As (III) to As (V), each bacterial colony was seeded in 5 ml of R2A medium, and then the cells were inoculated in to the R2A medium with 1 mM of As (III) at 25°C. After 3 days, the bacterial strains that have a capability of As (III) oxidation were preferentially screened by using the silver nitrate ($AgNO_3$) method (Dey *et al*., 2016; Kumari *et al*., 2019) and then were quantitatively measured by using the LC-ICP-MS (Akter *et al.*, 2005). Thus, we succeed to isolate the strain C25, which had a higher oxidation capability of As (III) among the single colonies obtained. After 12 h of cultivation, 1 mM of As (III) was completely oxidized to As (V) (data not shown).

Genomic DNA was obtained from the strain C25 using Maxwell 16 DNA purification kits (Promega) according to the manufacturer's instructions. The genomic DNA was sequenced at Macrogen, Inc., using an Illumina HiSeq X Ten sequencer with 151-bp paired-end reads. The resulting 4,155,450,540 bp with 27,519,540 total reads were trimmed and assembled using SOAP*denovo*. The draft genome of strain C25 consisted of 34 contigs with a total length of 5,191,050 bp (read N50, 431,687) and an average length of 152,677 bp (longest config of 1,269,555 and shortest contig of 1,094 bp). Aragon (v1.2.36) and Barrnap software (v0.6) were used to predict tRNAs and rRNAs (5S rRNAs, 16S rRNAs, and 23S rRNAs), respectively (Lagesen *et al*., 2007). Genome annotation was performed using the Prokaryotic Genome Annotation Pipeline (Tatusova *et al.*, 2016). Default parameters were employed for all software unless otherwise specified.

Genome features of *Methylobacterium brachythecii* C25 are presented in Table 1. The assembled genome sequence coverage was 800.5x and the genomic GC content was 66.03%. A total of 4,975 coding DNA sequences (CDSs), 3 rRNA (1 each of 5S rRNA, 16S rRNA, 23S rRNA), 46 tRNA, 1 tmRNA, 4 non-coding RNA, and 75 pseudo genes were predicted. Identification of this bacterium was performed through 16S rRNA gene amplification, which showed 98.72% similarity with *Methylobacterium brachythecii* 99b^T (Tani and Sahin, 2013).

Table 1. Genome features of *Methylobacterium brachythecii* C25

Genome features	Value
Genome size (bp)	5,191,050
G + C content(%)	66.03
No. of rRNAs (5S, 16S, 23S)	3 (1, 1, 1)
No. of tRNAs	49
No. of ncRNAs (noncoding)	4
Protein coding genes	4,975
Pseudo-genes	75

Strain C25 has two types of arsenite oxidase (Aio) genes (locus number: HCU64_21125, HCU64_21130) that may lead to oxidize As (III) to As (V). The genome also contains several stress resistance genes to As (III) such as arsenite resistance transcriptional regulator (HCU64_03160), arsenite membrane efflux pump (HCU64_16965, HCU64_21100, HCU64_22845), arsenite resistance protein (HCU64_16965, HCU64_21705, HCU64_22215). These defensive strategies possessed by this strain may allow it to survive in As (III)-contamination environment.

Nucleotide sequence accession number

The genome sequence and raw sequencing reads for strain C25 were deposited under GenBank accession number JAAUVI010000000, BioProject accession number PRJNA 615742, BioSample accession number SAMN14466051, and Sequence Read Archive (SRA) accession number SRR 22163625. Strain C25 was deposited in Korean Agricultural Culture Collection (KACC 19855).

적 요

중금속 오염지역으로부터 분리한 비소 산화 미생물 *Methylobacterium* 속 균주 C25의 유전체를 분석하였다. 이 세균은 독성이 강한 아비산염(3가 비소, As III)를 비산염(5가 비소, As V)로 산화시키며 비소의 독성을 저감할 수 있는 호기성 미생물이다. 유전체의 크기는 5,191,050 bp이며 G + C 함량은 66.03% 로 나타났다. 이 유전자는 4,975개의 단백질을 암호화

하는 서열을 비롯하여, 49개의 운반 RNA, 3개의 리보솜 RNA, 4개의 비번역 RNA, 75개의 유사유전자를 포함한다. 아비산염을 비산염으로 산화시킬 수 있는 2가지 종류의 아비산염 산화 효소(Aio, arsenite oxidase gene)를 보유하고 있다. 이러한 결과를 바탕으로, C25 균주는 아비산염을 산화하고, 아비산염 독성에 의한 스트레스를 잘 견딜 수 있는 것으로 예측된다.

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Conflict of Interest

The authors have no conflict of interest to report.

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