# Draft genome sequence of *Zhongshania marina* DSW25-10<sup>T</sup> isolated from seawater

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# 해수에서 분리된 *Zhongshania marina* DSW25-10<sup>T</sup>의 유전체 서열분석

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The draft genome sequencing for *Zhongshania marina* DSW25- $10^{T}$ , isolated from deep seawater of East Sea in Korea, was performed using Illumina HiSeq platform. As a result, the draft genome was comprised of a total length of approximately 4.08 Mbp with G+C content of 49.0%, and included a total of 3,702 protein-coding genes, 3 rRNA genes, 39 tRNA genes, 4 noncoding RNA genes, and 36 pseudogenes. In addition, the metabolic pathways of aliphatic and aromatic compounds were identified. In light of these metabolic pathways, *Zhongshania marina* DSW25- $10^{T}$  is expected to be a useful bioremediation resource.

**Keywords:** *Zhongshania marina* DSW25-10<sup>T</sup>, draft genome sequence, Illumina HiSeq

The genus *Zhongshania* in the class *Gammaproteobacteria* was first described by Li *et al.* (2011), and currently comprises 4 type species and an invalid published species: *Z. antarctica* (Li *et al.*, 2011), *Z. goukunii* (Li *et al.*, 2011), *Z. aliphaticivorans* (Lo *et al.*, 2014), *Z. borealis* (Jang *et al.*, 2011; Lo *et al.*, 2014), and '*Z. ponticola*' (Park *et al.*, 2018). These species were isolated from marine environments, such as coastal attached ice, seawater and marine sediment, and characterized as Gram-

negative, catalase-, and oxidase-positive, aerobic, and rod-shaped motile by single polar flagellum. Especially, *Z. aliphaticivorans*, isolated from crude oil contaminated sea-tidal flats, could be able to degrade aliphatic hydrocarbons, and identified the alkane 1-monooxygenase coding genes, catalyzing *n*-alkanes to fatty alcohols, by full genome sequencing (Jia *et al.*, 2016). In addition, the genome sequence of the strain *Zhongshania* sp. ZX-21 has been determined (PQGG00000000, unpublished).

The Zhongshania marina DSW25-10<sup>T</sup> was isolated from 200~500 m deep seawater, using a standard dilution plating method on marine agar 2216 (MA; Difco). For analysis of the genome sequence, the cells were incubated at 25°C in marine broth 2216 (MB; Difco) for 5 days and the genomic DNA was extracted using MagAttract HMW DNA kit (Qiagen). The genome was sequenced using Illumina HiSeq platform by Macrogen Inc. The *de novo* assembly was performed by SPAdes (version 3.10.0) (Bankevich et al., 2012). The potential contamination of the draft genomes was assessed using ContEst16S (Lee et al., 2017). The total of 46 contigs were obtained with N50 length of 170,247 bp and 150.6 × sequencing depth of coverage. The draft genome size was 4,084,538 bp with G + C content of 49.0%. Genome annotation was conducted by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (http://www. ncbi.nlm.nih.gov/genome/annotation prok/) and BlastKOALA

(Kanehisa *et al.*, 2016). A total of 3,702 protein-coding genes, 3 rRNA genes (5S, 16S, and 23S), 39 tRNA genes, 4 non-coding RNA genes, and 36 pseudogenes were predicted (Table 1).

The other strains of genus Zhongshiania have three to four alkane 1-monooxygenases whereas the strain DSW25-10<sup>T</sup> has one alkane 1-monooxygenases and 44% amino acid sequence identity with that (WP 062384540) of Z. aliphaticivorans. The draft genome also contained degradation metabolisms of aromatic compounds. Phenol hydroxylase gene cluster dmpKLMNOP for benzene degradation pathway and catechol meta cleavage pathway gene clusters xylEFGHIJKQ and mhpDEF were found. Associated with toluene degradation pathway, toluene monooxygenase gene cluster tmoABCDEF and aryl-alcohol dehydrogenase were found, but benzaldehyde dehydrogenase (NAD) was not found. Benzene degradation pathway was known that had two primary pathways in first oxidation step, conversion of benzene to phenol by benzene monooxygenase or toluene monooxygenase and benzene to cis-dihydrobenzenediol by benzene 1,2-dioxygenase (Zamanian and Mason, 1987; Tao et al., 2004; Choi et al., 2013). The draft genome of Zhongshania marina DSW25-10<sup>T</sup> did not include benzene monooxygenase and benzene 1,2-dioxygeanse, but toluene monooxygenase gene cluster was shown comparatively high amino acid sequence similarity with Pseudomonas mendocina KR1 and Ralstonia picketti PKO1, known as oxidize benzene to phenol by toluene monooxygenase. Based on this, it is assumed that Zhongshania marina DSW25-10<sup>T</sup> might oxidize benzene to phenol by toluene monooxygenase, and then phenol is converted to catechol through phenol hydroxylases.

The Zhongshania marina DSW25-10<sup>T</sup>, containing degra-

Table 1. Genome features of Zhongshania marina DSW25-10<sup>T</sup>

Genome features	Value
No. of contigs	46
Depth (×)	150.6
Genome size (bp)	4,084,538
G + C content (%)	49.0
Protein-coding genes	3,702
tRNA genes	39
rRNA genes (5S, 16S, 23S)	3 (1, 1, 1)
Non-coding RNA genes	4
Pseudogenes	36

dation metabolisms of saturated and aromatic hydrocarbons, is expected to be a useful biological resource for bioremediation of oil-polluted marine environments.

#### Nucleotide sequence accession numbers

The strain *Zhongshania marina* DSW25-10<sup>T</sup> is available at KCCM 43273 and JCM 17372. The draft genome sequence is accessible in GenBank under the accession number RHGB 00000000. The version described in this paper is version RHGB01000000.

### 적 요

이 연구에서는 Illumina Hiseq platform을 사용하여 동해 심층 해양수로부터 분리된 Zhongshania marina DSW25-10<sup>T</sup>의 유전체 염기서열 해독을 수행하였다. 그 결과, 유전체는 대략 4.08 Mbp의 길이 및 49.0%의 G+C 함량으로 구성되었고, 전체 3,702개의 단백질 암호 유전자, 3개의 rRNA 유전자, 39개의 tRNA 유전자, 4개의 non-coding RNA 유전자 및 36개의 위유전자(pseudogenes)가 확인되었다. 또한, 지방족 및 방향족화합물의 대사 경로가 확인되었다. 이러한 대사 경로들로 비추어 Zhongshania marina DSW25-10<sup>T</sup>는 유용한 생물 정화자원으로 사용될 수 있을 것으로 기대된다.

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#### References

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, *et al.* 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **19**, 455–477.

Choi EJ, Jin HM, Lee SH, Math RK, Madsen EL, and Jeon CO. 2013.
Comparative genomic analysis and benzene, toluene, ethylbenzene, and o-, m-, and p-xylene (BTEX) degradation pathways of Pseudoxanthomonas spadix BD-a59. Appl. Environ. Microbiol.

- 79, 663-671.
- Jang GI, Hwang CY, Choi HG, Kang SH, and Cho BC. 2011.
  Description of Spongiibacter borealis sp. nov., isolated from Arctic seawater, and reclassification of Melitea salexigens Urios et al. 2008 as a later heterotypic synonym of Spongiibacter marinus Graeber et al. 2008 with emended descriptions of the genus Spongiibacter and Spongiibacter marinus. Int. J. Syst. Evol. Microbiol. 61, 2895–2900.
- **Jia B, Jeong HI, Kim KH, and Jeon CO.** 2016. Complete genome of *Zhongshania aliphaticivorans* SM-2<sup>T</sup>, an aliphatic hydrocarbon-degrading bacterium isolated from tidal flat sediment. *J. Biotechnol.* **226**, 22–23.
- Kanehisa M, Sato Y, and Morishima K. 2016. BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. J. Mol. Biol. 428, 726–731.
- Lee I, Chalita M, Ha SM, Na SI, Yoon SH, and Chun J. 2017.
  ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* 67, 2053–2057.
- Li HJ, Zhang XY, Chen CX, Zhang YJ, Gao ZM, Yu Y, Chen XL, Chen B, and Zhang YZ. 2011. Zhongshania antarctica gen. nov., sp.

- nov. and *Zhongshania guokunii* sp. nov., gammaproteobacteria respectively isolated from coastal attached (fast) ice and surface seawater of the Antarctic. *Int. J. Syst. Evol. Microbiol.* **61**, 2052–2057
- Lo N, Kang HJ, and Jeon CO. 2014. Zhongshania aliphaticivorans sp. nov., an aliphatic hydrocarbon-degrading bacterium isolated from marine sediment, and transfer of Spongiibacter borealis Jang et al. 2011 to the genus Zhongshania as Zhongshania borealis comb. nov. Int. J. Syst. Evol. Microbiol. 64, 3768–3774.
- Park S, Park JM, Lee JS, and Yoon JH. 2018. *Zhongshania ponticola* sp. nov., a novel lipolytic bacterium of the class *Gammaproteobacteria* isolated from seawater. *Arch. Microbiol.* **200**, 1177–1182.
- Tao Y, Fishman A, Bentley WE, and Wood TK. 2004. Oxidation of benzene to phenol, catechol, and 1,2,3-trihydroxybenzene by toluene 4-monooxygenase of *Pseudomonas mendocina* KR1 and toluene 3-monooxygenase of *Ralstonia pickettii* PKO1. *Appl. Environ. Microbiol.* 70, 3814–3820.
- **Zamanian M and Mason JR.** 1987. Benzene dioxygenase in *Pseudomonas putida*. Subunit composition and immuno-cross-reactivity with other aromatic dioxygenases. *Biochem. J.* **244**, 611–616.