


Complete genome sequence analysis of *Photobacterium atrarenae* GJH2-4 reveals its genetic capability for benzene degradation isolated from marine environment

Minji Kim and Soo-Je Park* 

Department of Biology, Jeju National University, Jeju 63243, Republic of Korea

해양환경으로부터 분리된 벤젠 분해능을 지닌 *Photobacterium atrarenae* GJH2-4의 전장유전체 특성

김민지 · 박수제* 

제주대학교 생물학과

(Received July 19, 2022; Revised August 10, 2022; Accepted August 11, 2022)

The genus *Photobacterium* belongs to family *Vibrionaceae*, order *Vibrionales*, and phylum *Pseudomonadota*. It is known as a Gram-stain-negative and facultative aerobe that is motile. Members of the genus *Photobacterium* including pathogens have various life cycles. In this study, we isolated a strain GJH2-4 belonging to the genus *Photobacterium* from a marine sediment of Jeju Island, South Korea. Its complete genome sequence was found to have three circularized chromosomes. After *de novo* assembling, its total size and G + C content were about 5.41 Mb and 53.0%, respectively. The three chromosomes encoded 4,794 protein-coding sequences, 34 ribosomal RNAs, and 153 transfer RNA genes. Unexpectedly, the genome of strain GJH2-4 contained genes for benzene degradation, complete denitrification, dissimilatory nitrate reduction, assimilatory sulfate reduction, flagellar assembly, and vitamins biosynthesis based on its genetic functional characteristics. Furthermore, its genome encoded cytochrome *cbb*₃; oxidases known to have a high affinity for oxygen. Finally, we discovered that strain GJH2-4 could contribute to nutrient cycling in their habitats. Our findings might facilitate a better understanding of various capabilities of species in genus *Photobacterium*.

Keywords: *Photobacterium*, benzene, complete genome, marine

Generally, halophilic microorganisms (i.e., halophiles) require a salt concentration of at least 0.5% (w/v, NaCl) in order to grow. In addition, some halophiles (e.g., moderate halophiles) can grow at higher NaCl concentrations (> 5% w/v, NaCl). These extremely or moderately halophiles have specific adapted strategies including stable active site or promotion of subunit interactions for their enzymes (DasSarma and DasSarma, 2015), indicating that these halophiles are excellent sources of enzymes due to their ability to perform stable functions under extreme conditions and their applicability to diverse biotechnologies such as food processing and industrial bioconversion (Patel and Saraf, 2015). This study investigated culturable halophilic-bacterial diversity using a slightly high salt concentration of 4 or 5% (w/v, NaCl) than seawater. To isolate bacterial cells, a sample of the marine sediment soil (33°27'15" N, 126°18'26"E) was collected with a sterile spatula and promptly transported to the laboratory at 4°C with an icepack. The sample was then slurred by autoclaved-natural seawater and finally adjusted to 4 or 5% NaCl (w/v). The slurry mixture was serially diluted (ten-fold) to 10⁻⁴. Sequentially, 100 µl of the aliquot from the final diluted sample (10⁻⁴) was spread onto marine agar (BD Difco™) plates adjusted to 4 or 5% (w/v, NaCl; named adjusted-salt marine agar, ASM agar) and incubated at 30°C for one

*For correspondence. E-mail: sjpark@jejuu.ac.kr;
Tel.: +82-64-754-3524; Fax: +82-64-756-3541

week under aerobic conditions. After the first-round cultivation, several strains were selected based on colony morphology and color under naked eyes and transferred to new ASM agar plates. To obtain a purified single colony, colonies were successively transferred to new ASM agar plates at least five times. Finally, one isolated yellowish colony designed as GJH2-4 was collected at 4% (w/v) NaCl culture condition. The isolated strain GJH2-4 was deposited at the Korean Culture Center for Microorganisms (KCCM) (deposit number: KCCM 43405).

To analyze its phylogenetic and taxonomic positions, genomic DNA of strain GJH2-4 was extracted using a Monarch[®] Genomic DNA Purification Kit (New England Biolabs, Inc.). Its 16S ribosomal RNA gene sequence was then amplified and sequenced according to the previous study (Kim *et al.*, 2020). It was found that strain GJH2-4 was most closely related to *Photobacterium atrarenae* M3-4^T (HM452945, 99.6% 16S rRNA gene sequence similarities) (Kim *et al.*, 2011) using EzBioCloud server (<https://www.ezbiocloud.net/>). The genus *Photobacterium* of the order *Vibrionales* and the type species, *Photobacterium phosphoreum*, were reclassified by Beijerinck (1889) (formerly named as *Micrococcus phosphoreus*; Cohn, 1878). Members of the genus are Gram-stain-negative, facultative aerobic, motile, and widely distributed in various marine associated environments such as seawater and marine animals (Urbanczyk *et al.*, 2011). In addition, some strains belonging to the genus *Photobacterium* are pathogens having high virulence potentials (Matanza and Osorio, 2020; Pira *et al.*, 2022).

Whole-genome sequencing was performed at DNA Link, Inc. using a Pacific Biosciences RSII instrument (Pacific Biosciences). A single SMRT cell produced a total of 1.7 Gb (about 317 X in depth) in 312,782 polymerase reads that passed filtering. Finally, we obtained three contigs closed using RS

HGAP assembly (v3.0). Genome completeness and quality for assembled chromosomes were estimated with BUSCO (Manni *et al.*, 2021). Resulting assembled contigs were annotated with Prokka (v1.14.5) installed stand-alone and NCBI Prokaryotic Genome Annotation Pipeline (PGAP) using GeneMarkS-2+ version 5.2 with the best-placed reference protein method (Angiuoli *et al.*, 2008). In addition, to assign functional characteristic, protein sequences were predicted using Clusters of Orthologous Groups (COGs) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database references as described previously (Kim *et al.*, 2020). Finally, the length of the three circularized chromosomes of strain GJH2-4 was about 5.41 Mb with G + C content of 53.0%. BUSCO estimation showed 100% genome completeness.

The complete genomes of strain GJH2-4 estimated by Prokka had 4,984 predicted genes, 34 ribosomal RNAs, and 153 transfer RNA genes (Table 1). Based on the phylogenomic tree constructed with Type (strain) Genome Server (TYGS) (Meier-Kolthoff and Göker, 2019), strain GJH2-4 belonged to genus *Photobacterium* (Fig. 1), supporting the result for 16S rRNA gene phylogeny analysis. However, due to no genome sequence of *Photobacterium atrarenae* M3-4^T, we were unable to conduct digital DNA-DNA Hybridization (DDH) estimated by Genome-to-Genome Distance Calculator (GGDC) (Meier-Kolthoff *et al.*, 2013) in this study.

Resultant COGs revealed that the category of amino acid transport and metabolism (E, 9.94%) was the most dominant one in the genome, followed by transcription category (K, 8.17%) and the category of translation, ribosomal structure, and biogenesis (J, 7.85%) (Fig. 2).

KEGG analysis (n = 4,794) showed that complete chromosomes of strain GJH2-4 harbored genes for central metabolisms

Table 1. General features of the genome of *Photobacterium atrarenae* GJH2-4

Genome features	Chromosome 1	Chromosome 2	Chromosome 3
Genome length (Mb)	3.46	1.86	0.09
GC content (%)	53.5	52.3	50.9
No. of total predicted genes*	3,235	1,655	94
No. of total coding sequences*	3,054	1,648	92
No. of rRNA (23S, 16S, 5S)*	34 (11, 11, 12)	0	0
No. of tRNA*	146	7	0

* Estimated by Prokka (v.1.14.5).

such as glycolysis, gluconeogenesis, glyoxylate cycle as an anabolic tricarboxylic acid cycle, and biosynthesis and degradation for glycogen. In addition, the genome contained genes for assimilatory sulfate reduction (*cysNDCHJI*), dissimilatory nitrate reduction (nitrate to ammonia, *napAB*, *nirBD*, and

nrfAH), and denitrification (nitrate to nitrogen, *napAB*, *nirK*, *norBC*, and *nosZ*). Strain GJH2-4 genome harbored a complete gene cluster for benzene degradation, which could degrade benzene to acetyl-CoA via catechol. Lots of the ABC transporters (n = 107) were identified in the genome, including phosphate,

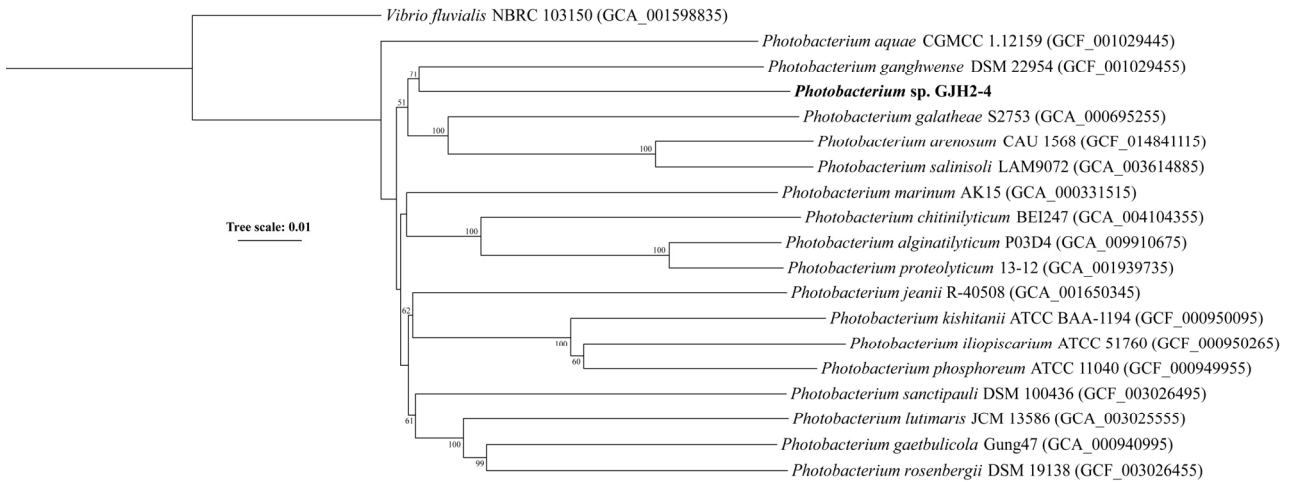


Fig. 1. Tree inferred with FastME 2.1.6.1 from GBDP distances calculated from genome sequences. Branch lengths are scaled in terms of GBDP distance formula d_5 . Numbers above branches are GBDP pseudo-bootstrap support values > 50% from 100 replications. The tree was rooted at the midpoint by *Vibrio fluvialis* NBRC 103150 (GCA_001598835).

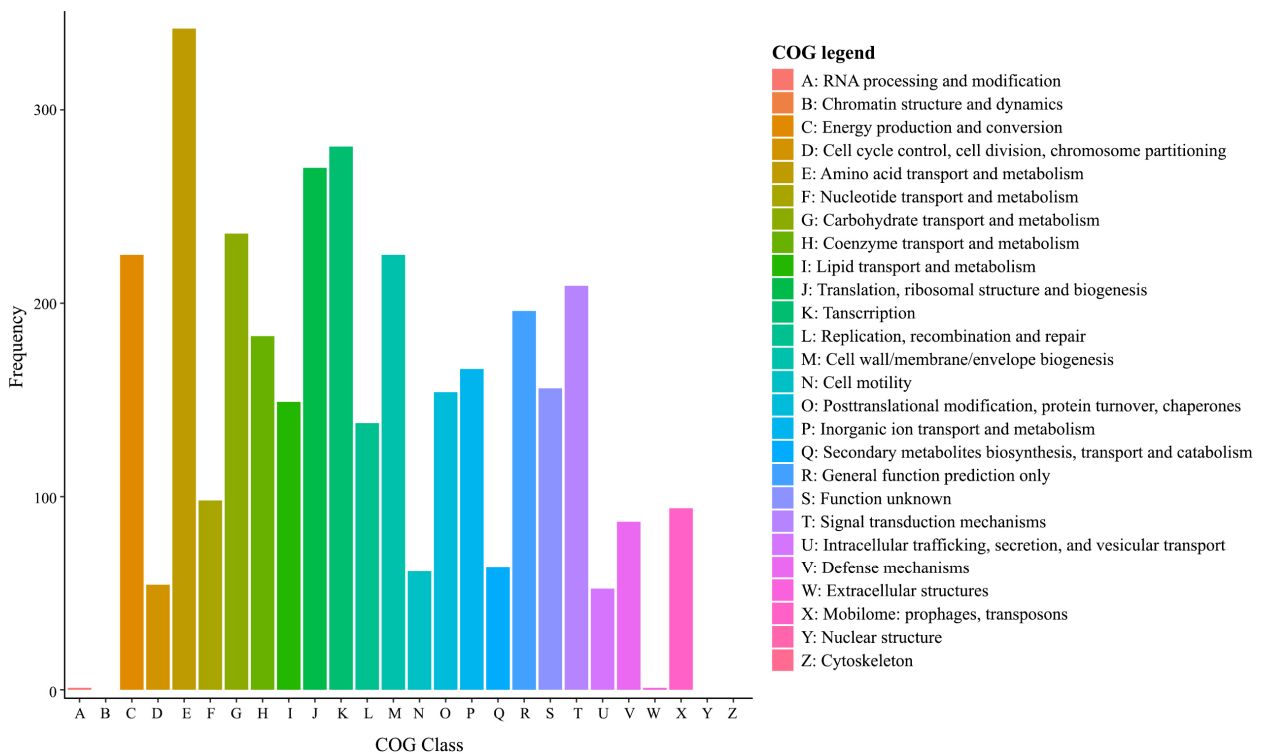


Fig. 2. Distribution of clusters of orthologous groups (COGs) for strain GJH2-4 genome. The x-axis represents single letter for COG class and the y-axis represents gene frequency. Each COG functional class with single letter is presented in the right panel.

phosphonate, amino acids, phospholipid, oligopeptide, glycine betaine, and osmoprotectant. Interestingly, vitamin B₁₂ (also called cobalamin) transporter (BtuFCD) was found in the genome with complete genes for cobalamin biosynthesis. These results imply that strain GJH2-4 contributes to nitrogen and carbon cycles in marine sediment (Herbert, 1999) with a possibility for growth factors supply to other organisms (Sañudo-Wilhelmy *et al.*, 2014). However, contributions of the genus *Photobacterium* in the family *Vibrionaceae* are largely unknown. In the same class of *Gammaproteobacteria*, the cobalamin-mediated metabolic process of the family *Psychromonadaceae* has been analyzed based on ecogenomics (Jin *et al.*, 2022).

In addition, the genome of strain GJH2-4 harbored many genes for two-component system (n = 115), quorum sensing (n = 47), biofilm formation (n = 131), pilus system (twitching motility and type IV pilus, n = 18), and nearly the entire flagellar assembly (n = 41). The genome harbored *cbb₃*-type (cytochrome *c* oxidase) as a high oxygen affinity under low-oxygen conditions (Pitcher and Watmough, 2004). Taken together, these genetic potentials indicate that strain GJH2-4 might have ecophysiological dominant position against other competitors in its habitats.

Nucleotide sequence accession number

The complete genome sequence of strain GJH2-4 (= KCCM 43405) has been deposited at DDBJ/ENA/GenBank under accession numbers CP101508 to CP101510.

적 요

Pseudomonadota 문, *Vibrionales* 목에 속하는 *Photobacterium* 속은 그람 음성, 통성 호기성 및 운동성을 지닌 것으로 알려져 있다. 또한, 병원균을 포함한 *Photobacterium* 속 세균들은 다양한 생활사를 가지고 있다. 본 연구에서는 제주의 해양 퇴적물에서 *Photobacterium* 속의 GJH2-4 균주를 분리하여 완전한 게놈 서열을 분석하였다. 균주의 유전체 염기서열은 새로이 (*de novo*) 조립되어, 총 3개의 완전 전장유전체가 확인되었으며, 전체 게놈 크기와 G + C 함량은 각각 약 5.41 Mb와 53.0%였다. 3개의 완전 전장유전체는, 4,794개의 단백질을 암호화하는 서열을 비롯하여, 34개의 리보솜 RNA, 153개의 운반 RNA 유전자가 확인되었다. 유전체 분석 결과, GJH2-4 균주

의 게놈은 벤젠 분해, 완전 탈질화, 이화적 질산염 환원, 동화적 황산염 환원, 편모 조립과 다양한 비타민 생합성과 관련된 유전자를 지니고 있다. 또한, 저산소 조건에서 높은 산소 친화력을 가지는 *cbb₃* 타입의 시토크롬 산화효소를 암호화하고 있다. 본 연구를 통하여, GJH2-4 균주가 물질 순환에 기여하는 것을 발견하였으며, 이는 *Photobacterium* 속에 속하는 세균의 보다 다양한 능력에 대한 이해에 도움이 될 것이다.

Acknowledgments

This research was supported by the 2022 scientific promotion program funded by Jeju National University.

Conflict to Interest

The authors have no conflict of interest to report.

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