Genome sequence of *Lysobacter dokdonensis* DS-58<sup>T</sup>, a gliding bacterium isolated from soil in Dokdo, Korea

Min-Jung Kwak<sup>1</sup>, Soon-Kyeong Kwon<sup>1</sup>, Jung-Hoon Yoon<sup>2</sup> and Jihyun F. Kim<sup>1*</sup>

**Abstract**

*Lysobacter dokdonensis* DS-58, belonging to the family *Xanthomonadaceae*, was isolated from a soil sample in Dokdo, Korea in 2011. Strain DS-58 is the type strain of *L. dokdonensis*. In this study, we determined the genome sequence to describe the genomic features including annotation information and COG functional categorization. The draft genome sequence consists of 25 contigs totaling 3,274,406 bp (67.24 % G + C) and contains 3,155 protein coding genes, 2 copies of ribosomal RNA operons, and 48 transfer RNA genes. Among the protein coding genes, 75.91 % of the genes were annotated with a putative function and 87.39 % of the genes were assigned to the COG category. In the genome of *L. dokdonensis*, a large number of genes associated with protein degradation and antibiotic resistance were detected.

**Keywords:** Dokdo, *Xanthomonadaceae*, Protease, Peptidase, Soil bacterium

**Introduction**

The genus *Lysobacter* was firstly described by Christensen and Cook in 1979 as high G + C Gram-negative bacterium with gliding motility [1]. In the past, *Lysobacter* species were classified as “unidentified myxobacters” due to their high G + C ratio and gliding motility. However, the genus *Lysobacter* has features distinctive from myxobacteria and had been proposed as a new genus of *Gammaproteobacteria*. *Lysobacter* species are ubiquitous and have been found in a variety of environments such as soil, water, and the rhizosphere. Currently, more than 30 *Lysobacter* species were registered in the GenBank taxonomy database and among them, 28 species have been validly published [2]. Some of the *Lysobacter* species were known to produce several kinds of lytic enzymes and antibiotics [3] and have an antimicrobial activity against plant pathogens [4]. Moreover, several *Lysobacter* species are known to produce bioactive natural products such as cyclodepsipeptide, cyclic lipodepsipeptide, cepham-type β-lactam, and poly cyclic tetramate macrolactam [5]. Despite their ubiquitous distribution, many identified species, and possible usefulness as a biocontrol agent, deciphered *Lysobacter* genomes are relatively limited. Here, we present the genome sequence and the genomic information of *Lysobacter dokdonensis* DS-58<sup>T</sup> (KCTC 12822<sup>T</sup> = DSM 17 958<sup>T</sup>), which is the type strain of the species.

**Organism information**

**Classification and features**

*L. dokdonensis* DS-58<sup>T</sup> is a Gram-staining-negative, non-motile, and rod-shaped bacterium and was isolated from the soil sample in Dokdo, an island in the East Sea, Korea, in 2011 [6]. *L. dokdonensis* DS-58 grows at the temperature range of 4 to 38 °C, the pH range of 6.0 to 8.0, and the NaCl concentration of 0 to 0.5 % (w/v) [6]. Colony size of *L. dokdonensis* DS-58 is about 1.0 – 2.0 mm on nutrient agar medium and the cell size is 1.0–5.0 μm long and 0.4–0.8 μm wide [6] (Fig. 1). *L. dokdonensis* DS-58 can assimilate dextrin, Tween 40, maltose, α-ketobutyric acid, alaninamide, L-alanine, L-alanyl glycine, and L-glutamic acid as a carbon source [6]. Minimum information about a genome sequence (MIGS) for *L. dokdonensis* DS-58 is described in Table 1. Phylogenetically, *L. dokdonensis* DS-58 belongs to the family *Xanthomonadaceae* of the class *Gammaproteobacteria*, and the 16S rRNA gene showed the highest
sequence similarity (96.93 %) with \textit{L. niastensis} GH41-7. However, a phylogenetic tree based on the 16S rRNA gene showed that the strain DS-58 is located in the deep branch of the genus \textit{Lysobacter} (Fig. 2).

**Genome sequencing information**

**Genome project history**
The genome sequencing and analysis of \textit{L. dokdonensis} DS-58 were performed by the Laboratory of Microbial Genomics and Systems/Synthetic Biology at Yonsei University using the next generation sequencing. The genomic information was deposited in the GenBank (Accession number is JRKJ00000000). Summary of the genome project is provided in Table 2.

**Growth conditions and genomic DNA preparation**
\textit{L. dokdonensis} DS-58 (accession numbers of culture collection: KCTC 12822 = DSM1 7958) was routinely cultured on nutrient medium at 30 °C. Strain DS-58 forms light yellow colored colonies with average 1.0–2.0 mm of diameter in 5 days (Table 1) [6]. For the genome

| MIGS ID | Property            | Term                                                                 | Evidence code$^a$ |
|---------|---------------------|----------------------------------------------------------------------|-------------------|
| MIGS-6  | Habitat             | Soil                                                                 | TAS [6]           |
| MIGS-6.3| Salinity            | 0–0.5 % NaCl (w/v)                                                   | TAS [6]           |
| MIGS-22 | Oxygen requirement  | Aerobic                                                              | TAS [6]           |
| MIGS-15 | Biotic relationship | Free-living                                                         | TAS [6]           |
| MIGS-14 | Pathogenicity       | Unknown                                                             | NAS               |
| MIGS-4  | Geographic location | Republic of Korea                                                   | TAS [6]           |

Table 1 Classification and general features of \textit{Lysobacter dokdonensis} DS-58$^1$ according to the MIGS recommendations [24]

$^a$ TAS: Traceable Authoritative Source; NAS: Not Assessed
sequencing, single colony of *L. dokdonensis* DS-58 was inoculated in nutrient medium and incubated in the shaking incubator at 30 °C. Genomic DNA was extracted using chemical and enzymatic method as described in Molecular Cloning, A Laboratory Manual [7]. Cell lysis was conducted using sodium dodecyl sulfate and proteinase K. From the cell lysate, genomic DNA was purified using phenol:chloroform, precipitated using isopropanol, and finally eluted into Tris-EDTA buffer.

**Genome sequencing and assembly**

For the whole genome shotgun sequencing, a library with 500-bp insert size was prepared and paired-end genome sequencing was performed with HiSeq2000 of

![Fig. 2 Neighbour-joining tree of the type species of the genus *Lysobacter*. Neighbor-joining tree based on the 16S rRNA gene sequence was constructed using MEGA 5. The evolutionary distances were calculated using Jukes-Cantor model and phylogenetic tree was generated based on the comparison of 1,379 nucleotides. Bootstrap values (percentages of 1,000 replications) greater than 50 % are shown at each node and *Xanthomonas campestris* ATCC 33913 (AE008922) were used as an out-group. The scale bar represents 0.005 nucleotide substitutions per site. Accession numbers of the 16S rRNA gene are presented in the parentheses. *species whose genome has been sequenced.*]
the Illumina/Solexa platform (Macrogen, Inc., South Korea). Sequence trimming was conducted using CLC Genomics Workbench 5.1 (CLC bio, Qiagen, Netherlands) with parameters of 0.01 quality score and none of the ambiguous nucleotide. Sequence reads below 60 bp in length were discarded. After trimming, a total of 28,810,330 reads with an average read length of 95.8 bp were generated. 

**Genome annotation**

Structural gene prediction was conducted using Glimmer 3 [10] in RAST server [11] with automatic fixation of errors and frame shifts. Functional assignment of the predicted protein coding sequences (CDSs) was performed using AutoFact [12] with the results of BLASTP or RPS-BLAST with Uniref100, NR, COG, and Pfam databases. For the accurate annotation, the functional assignment results from the RAST server and BLAST were compared each other. When assignment of the gene function was not the same between the results from RAST and BLAST, an additional BLASTP search was performed with NR database at NCBI and the top-hit result was selected for the annotation.

**Genome properties**

The draft genome sequence of the strain DS-58 consists of 25 contigs and the sum of the contigs is 3,274,406 bp (G + C content 67.24 %) (Table 3 and Fig. 3). From the genome of the strain DS-58, 3,155 CDSs, 2 copies of ribosomal RNA operons, and 48 transfer RNAs were detected. Among the predicted CDSs, 2,436 CDSs were annotated with a putative function and 2,757 CDSs were

![Fig. 3 Circular representation of the draft genome of Lysobacter dokdonensis DS-58.](image-url)
assigned to a COG category. The numbers and percentages of COG assigned genes are shown in Table 4.

**Insights from the genome sequence**

Some *Lysobacter* species are known to produce the secondary metabolite with antimicrobial activities [13, 14]. In the genome of *L. dokdonensis* DS-58, biosynthetic gene clusters for a bacteriocin and an arylpolyene were detected. The structure of bacteriocin-biosynthetic gene cluster of DS-58 was similar to the one in *L. arsenicireis-tens* ZS79 and the structure of arylpolyene-biosynthetic gene cluster was similar to the one in *Xanthomonas camp-pestris* NCPPB 4392 (Fig. 4).

Table 4 Number of protein coding genes of *Lysobacter dokdonensis* DS-58 associated with the general COG functional categories

| Code | Value | %age | Description |
|------|-------|------|-------------|
| J    | 168   | 5.32 | Translation, ribosomal structure and biogenesis |
| A    | 5     | 0.16 | RNA processing and modification |
| K    | 164   | 5.20 | Transcription |
| L    | 120   | 3.80 | Replication, recombination and repair |
| B    | 1     | 0.03 | Chromatin structure and dynamics |
| D    | 34    | 1.08 | Cell cycle control, cell division, chromosome partitioning |
| Y    | 0     | 0.00 | Nuclear structure |
| V    | 60    | 1.90 | Defense mechanisms |
| T    | 232   | 7.35 | Signal transduction mechanisms |
| M    | 219   | 6.94 | Cell wall/membrane/envelope biogenesis |
| N    | 60    | 1.90 | Cell motility |
| Z    | 3     | 0.10 | Cytoskeleton |
| W    | 1     | 0.03 | Extracellular structures |
| U    | 96    | 3.04 | Intracellular trafficking, secretion, and vesicular transport |
| O    | 119   | 3.77 | Posttranslational modification, protein turnover, chaperones |
| C    | 142   | 4.50 | Energy production and conversion |
| G    | 90    | 2.85 | Carbohydrate transport and metabolism |
| E    | 184   | 5.83 | Amino acid transport and metabolism |
| F    | 57    | 1.81 | Nucleotide transport and metabolism |
| H    | 109   | 3.45 | Coenzyme transport and metabolism |
| I    | 114   | 3.61 | Lipid transport and metabolism |
| P    | 113   | 3.58 | Inorganic ion transport and metabolism |
| Q    | 55    | 1.74 | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 308   | 9.76 | General function prediction only |
| S    | 303   | 9.60 | Function unknown |
| -    | 398   | 12.61 | Not in COGs |

*The percentages are based on the total number of protein coding genes in the genome.*

In the genome of *L. dokdonensis* DS-58, a number of genes associated with proteolysis were detected that include 63 genes encoding peptidases and 33 genes encoding proteases. Microbial proteases are among the most important industrial enzymes due to their diverse activities and the genus *Bacillus* is major source of protease in the market [15, 16]. Results from the text mining of annotated gene products indicated that *L. dokdonensis* DS-58 has more genes encoding proteases and pepti-dases than other genome-sequenced *Lysobacter* species except for *L. antibioticus* ASM73109v1 and *L. capsici* AZ78. Moreover, in the genome of the strain DS-58, genes encoding 17 β-lactamases for degrading chemicals such as β-lactam antibiotics, biotin-biosynthetic proteins, and type IV fimbrial biogenesis proteins that could be involved in gliding motility were detected.

Distinct from other genera in the *Xanthomonadaceae*, *Lysobacter* spp. exhibit gliding motility [1]. Type IV pilus-associated bacterial motility is widespread in members of diverse taxa such as *Proteobacteriia, Bacteroidetes*, and *Fibrobacteres* [17] and known to be responsible for S-motility in *Myxococcus* and twitching motility in *Lysobacter* [18] as well as *Pseudomonas* and *Neisseria* [19]. Thus, there is a possibility that the gliding motility of *Lysobacter* is associated with type IV fimbriae. On the other hand, GltA, which is involved in A-motility of *Myxococcus xanthus* that best fits the definition of gliding motility [20], was detected in the genome of DS-58 (56 % identity with 88 % coverage).

*Lysobacter* species typically have been isolated from soil and water, but several studies indicated that *Lysobacter* species may survive in more diverse habitats of anaerobic or extreme-cold [21, 22]. A great diversity of secreted degrading enzymes such as proteases and β-lactamases may contribute to the adaptation of *Lysobacter* species to such diverse environments. Abundant genes encoding proteases and peptidases in the genome of DS-58 may contribute to the discovery of effective and commercially useful proteolytic enzymes. Moreover, in the genome of DS-58, dozens of genes involved in the biosynthesis of type IV fimbriae were detected. The mechanism of gliding motility has not yet been clearly revealed, and we expect that the genome information of DS-58 may contribute to the genetic analysis of bacterial gliding motility.

**Conclusions**

*L. dokdonensis* DS-58, the type strain of the species, is a soil bacterium isolated from Dokdo in Korea. Through a phylogenetic analysis of the 16S rRNA gene, *L. dokdo-nensis* is located in a deep branch of the genus *Lysobac-ter*. The genome sequence of *L. dokdonensis* DS-58 is comprised of 25 contigs of 3,274,406 bp with G + C content of 67.24 %. In the genome of DS-58, a total of 3,155
CDs were predicted and 87.39 % of the CDs were functionally assigned to COG categories. Dozens of genes associated with protein degradation and resistance to antibiotics were detected. Through the genome analysis of *L. dokdonensis* DS-58, we report that this soil bacterium harbors a large number of peptidases and proteases, which may represent a rich source of protein-degrading enzymes.

**Abbreviations**

COG: Clusters of Orthologous Groups; NR: Non-redundant; UniRef: UniProt Reference Clusters; Pfam: Protein families; SSPACE: SSARE-based Scaffolding of Pre-Assembled Contigs after Extension; IMAGE: Iterative Mapping and Assembly for Gap Elimination; RAST: Rapid Annotation using Subsystem Technology; AutoFACT: Automatic Functional Annotation and Classification Tool; BLAST: Basic Local Alignment Search Tool; RPS-BLAST: Reversed Position Specific-BLAST; MEGA: Molecular Evolutionary Genetics Analysis; MIGS: Minimum Information about a Genome Sequence; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeat.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

JFK conceived, organized and supervised the project, interpreted the results, and edited the manuscript. SKK prepared the high-quality genomic DNA and arranged the acquisition of sequence data. MJK performed the sequence assembly, gene prediction, gene annotation, analyzed the genome information, and drafted the manuscript. JHY provided the bacterium and its microscopic image. All of the authors read and approved the final version of the manuscript before submission.

**Acknowledgements**

This work was financially supported by the National Research Foundation (NRF-2011-0017670) of the Ministry of Science, ICT and Future Planning, Republic of Korea.

**Author details**

1. Department of Systems Biology and Division of Life Sciences, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-749, Republic of Korea.
2. Department of Food Science and Biotechnology, Sungkyunkwan University, Suwon, Republic of Korea.

Received: 21 March 2015 Accepted: 25 November 2015

**References**

1. Christensen P, Cook FD. Lysobacter, a new genus of nonfruiting, gliding bacteria with a high base ratio. Int J Syst Bacteriol. 1978;28:367–93.
2. Garrity GM, Lyons C. Future-proofing biological nomenclature. OMICS. 2003;7:311–3.
3. Fu E, Riesch D, Zhang Y. Identification and characterization of Lysobacter *Capsici* strain PG4: a new plant health-promoting rhizobacterium. J Plant Pathol. 2010;92:57–64.
4. Qian GL, Hu BS, Jiang YH, Liu QF. Identification and characterization of Lysobacter enzymogenes as a biological control agent against some fungal pathogens. Agr Sci China. 2009;8:68–75.
5. Xie Y, Wright S, Shen Y, Du L. Bioactive natural products from Lysobacter enzymogenes. Nat Prod Rep. 2012;29:17–87.
6. Oh KH, Kang SJ, Jung YT, Oh TK, Yoon JH. Lysobacter dokdonensis sp. nov., isolated from soil. Int J Syst Evol Microbiol. 2011;61:1089–93.
7. Green Michael R, Sambrook J. MOLECULAR CLONING A Laboratory Manual. fourth ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2012.
8. Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. Scaffolding pre-assembled contigs using SSPACE. Bioinformatics. 2011;27:578–9.
9. Tsi II, Otto TD, Berriman M. Improving draft assemblies by iterative mapping and assembly of short reads to eliminate gaps. Genome Biol. 2010;11:R41.
10. Salzberg SL, Delcher AL, Kasif S, White O. Microbial gene identification using interpolated Markov models. Nuclic Acids Res. 1998;26:65–48.
11. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST server: Rapid annotations using subsystems technology. BMC Genomics. 2008;9:75.
12. Koski LB, Gray MW, Lang BF, Burger G. AutoFACT: an automatic functional annotation and classification tool. BMC Bioinformatics. 2005;6:151.
13. Zhang J, Du LC, Liu FQ, Xu FF, Hu BS, Venturi V, et al. Involvement of both PKS and NRPS in antibacterial activity in Lysobacter enzymogenes *OH1-1*. Fems Microbiol Lett. 2014;355:170–6.
14. Puopolo G, Cimmino A, Palmieri MC, Giovannini O, Evidente A, Pertot I. Lysobacter capsici AZ78 produces cyclo(L-Pro-L-Tyr), a 2,5-diketopiperazine with toxic activity against sporangia of Phytophthora infestans and Plasmopara viticola. J Appl Microbiol. 2014;117:1168–80.

15. Abebe B, Abrahim S, Genet A, Hiwot G, Paulos K, Melese A. Isolation, optimization and characterization of protease producing bacteria from soil and water in Gondar town, North West Ethiopia. IJBVI. 2014;1:20–4.

16. Soundra JF, Ramya V, Neelam D, Suresh Babu G, Siddalingeshwara K, Venugopal N, et al. Isolation, production and characterization of protease from Bacillus sp. isolated from soil sample. J Microbiol Biotech Res. 2012;2:163–8.

17. Agrebì R, Wantel M, Brochier-Armanet C, Mignot T. An evolutionary link between capsular biogenesis and surface motility in bacteria. Nat Rev Microbiol. 2015;13:318–26.

18. Zhou X, Qian GL, Chen Y, Du LC, Liu FQ, Yuen GY. PiIG is Involved in the Regulation of Twitching Motility and Antifungal Antibiotic Biosynthesis in the Biological Control Agent Lysobacter enzymogenes. Phytopathology. 2015;105:1318–24.

19. Shi W, Sun H. Type IV pilus-dependent motility and its possible role in bacterial pathogenesis. Infection and Immunity. 2002;70:1–4.

20. Mauriello EMF, Mignot T, Yang ZM, Zusman DR. Gliding Motility Revisited: How Do the Myxobacteria Move without Flagella? Microbiol Mol Biol R. 2010;74:229–44.

21. Bae HS, Im WT, Lee ST. Lysobacter concrcticus sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. Int J Syst Evol Micr. 2005;55:1155–61.

22. Kimura T, Fukuda W, Sanada T, Imanaka T. Characterization of water-soluble brown pigment from Antarctic bacterium, Lysobacter oligotrophicus. J Biosci Bioeng. 2014;113:1389–1723.

23. Bijn K, Medema MH, Kazempour D, Fischbach MA, Breitling R, Takano E, et al. AntiSMASH 2.0—a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Res. 2013;41:W204–212.

24. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, et al. The minimum information about a genome sequence (MIGS) specification. Nat Biotechnol. 2008;26:541–7.

25. Woese CR, Kandler O, Wheelis ML. Towards a Natural System of Organisms—Proposal for the Domains Archaea, Bacteria, and Eucarya. P Natl Acad Sci USA. 1990;87:4576–9.

26. Garrity GM, Bell JA, Lilburn T. Phylum XIV. Proteobacteria phyl. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. Bergey’s Manual of Systematic Bacteriology, vol. 2. 2nd ed. New York: Springer; 2005. p. 1.

27. Garrity GM, Bell JA, Lilburn T. Class III. Gammaproteobacteria class. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. Bergey’s Manual of Systematic Bacteriology, vol. 2. 2nd ed. New York: Springer; 2005. p. 1.

28. Saddler GS, Bradbury JF. Order III. Xanthomonadales ord. nov. In: Garrity GM, Brenner DJ, Krieg NR, editors. Bergey’s Manual of Systematic Bacteriology, vol. 2. 2nd ed. New York: Springer; 2005. p. 63.

29. Saddler GS, Bradbury JF. Family I. Xanthomonadaceae. In: Garrity GM, editor. Bergey’s Manual of Systematic Bacteriology, vol. 2. 2nd ed. New York: Springer; 2005. p. 63.

30. Saddler GS, Bradbury JF. Lysobacter Christensen and Cook 1978, 372AL. In: Garrity GM, editor. Bergey’s Manual of Systematic Bacteriology, vol. 2. 2nd ed. New York: Springer; 2005. p. 95–101.

31. Maiti M, Cook FD. Lysobacter, a New Genus of Nonfruiting, Gliding Bacteria with a High Base Ratio. Int J Syst Evol Micr. 1978;28:367–93.

32. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene Ontology: tool for the unification of biology. Nat Genet. 2000;25:25–9.