

Complete genome sequence of *Bacillus licheniformis* 14ADL4 exhibiting resistance to clindamycin

Do-Won Jeong¹ , Byunghoon Lee², and Jong-Hoon Lee^{2*} 

¹Department of Food and Nutrition, Dongduk Women's University, Seoul 02748, Republic of Korea

²Department of Food Science and Biotechnology, Kyonggi University, Suwon 16227, Republic of Korea

Clindamycin 내성 *Bacillus licheniformis* 14ADL4의 유전체 염기서열

정도원¹  · 이병훈² · 이종훈^{2*} 

¹동덕여자대학교 식품영양학과, ²경기대학교 식품생물공학과

(Received May 23, 2018; Revised May 28, 2018; Accepted May 28, 2018)

Clindamycin resistant *Bacillus licheniformis* 14ADL4 was isolated from doenjang, a Korean high-salt-fermented soybean food. Strain 14ADL4 contains a single circular 4,332,232 bp chromosome with a G + C content of 45.86%. The complete genome of strain 14ADL4 includes *lmrA* and *lmrB* homologs may confer resistance to clindamycin.

Keywords: *Bacillus licheniformis*, clindamycin resistance, doenjang, starter

Bacillus licheniformis is one of the predominant bacterial species identified in fermented soybean foods of Korea (Kim *et al.*, 2009; Lee *et al.*, 2010). We considered the species as a potential starter candidate of doenjang, a Korean high-salt-fermented soybean food ripened at NaCl concentrations of > 12% (w/w), because of its salt tolerance on tryptic soy agar (TSA; Sigma-Aldrich) supplemented with 14% (w/v) NaCl and activities of amylase, protease, and lipase which can contribute to degrade macromolecules in soybeans (Jeong *et al.*, 2014, 2017). In the process of selecting *B. licheniformis* starter candidates with biosafety from our stock cultures, we identified strain 14ADL4 resistant to clindamycin (Jeong *et al.*, 2017). The minimum inhibitory concentration of clindamycin for strain 14ADL4

determined by the broth micro-dilution method according to the guidelines of the Clinical and Laboratory Standards Institute (2007) was 64 mg/L and the strain exhibited growth on TSA containing 30 mg/L clindamycin. The present study performed a complete genome analysis of strain 14ADL4 to shed light on the genetic background behind the phenotypic resistance to clindamycin.

Whole-genome sequencing was performed using the PacBio Single-Molecule Real-Time (SMRT) sequencing system by ChunLab, Inc. One contig was generated from a hybrid assembly of reads from the PacBio system (105,988 reads and 345.13 coverage) for *B. licheniformis* 14ADL4. The reads were assembled using CLC Genomics Workbench ver. 7.5.1 (CLC Bio) and CodonCode Aligner (CodonCode Co.). Gene prediction was performed using Glimmer 3 (Delcher *et al.*, 2007), followed by annotation through a search against the Clusters of Orthologous Groups (COG) database (Tatusov *et al.*, 1997).

The complete genome of strain 14ADL4 consists of a single circular 4,332,232 bp chromosome with a G + C content of 45.86% (Table 1). The genome is predicted to contain 4,419 protein coding sequences, 81 tRNA genes, and 24 rRNA genes. A total of 3,900 genes were functionally assigned to categories based on COG assignments. Gene category analysis shows that the majority of the genes are related to carbohydrate transport

*For correspondence. E-mail: jhl@kgu.ac.kr;
Tel.: +82-31-249-9656; Fax: +82-31-253-1165

Table 1. Genome features of *Bacillus licheniformis* 14ADL4

Features	Value
Genome size (bp)	4,332,232
GC content (%)	45.86
Open reading frames	4,419
CDS assigned by COG	3,900
rRNA genes	24
tRNA genes	81

and metabolism (329 genes [8.4%]), followed by transcription (319 genes [8.2%]) and amino acid transport and metabolism (312 genes [8.0%]).

Strain 14ADL4 has two potential lincomycin resistance genes, *lmrA* (designated BL14DL4_03907, putative HTH-type transcriptional regulator gene) and *lmrB* (BL14DL4_03548, lincomycin resistance protein gene). The *lmrAB* genes, which encode a transcriptional repressor and a multidrug efflux protein, have been reported to be involved in the resistance of *Bacillus subtilis* to puromycin and lincomycin (Murata *et al.*, 2003). Erythromycin ribosome methylase gene (*erm*) has been reported to confer resistance to clindamycin as well as erythromycin (Leclercq, 2002), while any homolog of *erm* was not found in the genome of strain 14ADL4. In this context, the *lmrAB* operon found in strain 14ADL4 may confer resistance to lincosamide antibiotics including clindamycin. Comparative genomic analysis of *B. licheniformis* 14ADL4 with clindamycin sensitive *B. licheniformis* strains will provide further genetic insight into the strain-specific clindamycin resistance of *Bacillus* species. Illumination of the clindamycin resistance ortholog for *B. licheniformis* will contribute to introduce a safety-approved *Bacillus* starter culture in food fermentations.

Nucleotide sequence accession number

B. licheniformis 14ADL4 has been deposited in the Korean Collection for Type Cultures under accession number KCTC 33983 and its complete genome sequence has been deposited in DDBJ/ENA/GenBank under accession number CP026673.

적 요

Clindamycin 내성 *Bacillus licheniformis* 14ADL4 균주는 된장으로부터 분리되었다. 14ADL4 균주의 유전체분석 결과, GC 함량 45.86%, 약 4.3 Mb 크기의 염색체가 확인되었고, 유전체 상의 *lmrA*와 *lmrB* 추정 유전자가 clindamycin 내성에 관

여할 것으로 예상된다.

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1D1A1B01011421 and NRF2016R1D1A1B03930239). This work was also supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through the Technology Commercialization Support Program funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA, 815003-3). Byunghoon Lee was supported by Kyonggi University's Graduate Research Assistantship 2018.

References

- Clinical and Laboratory Standards Institute.** 2007. Performance standards for antimicrobial susceptibility testing. Seventeenth informational supplement. CLSI, Wayne, PA, USA.
- Delcher, A.L., Bratke, K.A., Powers, E.C., and Salzberg, S.L.** 2007. Identifying bacterial genes and endosymbiont DNA with glimmer. *Bioinformatics* **23**, 673–679.
- Jeong, D.W., Jeong, M., and Lee, J.H.** 2017. Antibiotic susceptibilities and characteristics of *Bacillus licheniformis* isolates from traditional Korean fermented soybean foods. *LWT-Food Sci. Technol.* **75**, 565–568.
- Jeong, D.W., Kim, H.R., Jung, G., Han, S., Kim, C.T., and Lee, J.H.** 2014. Bacterial community migration in the ripening of doenjang, a traditional Korean fermented soybean food. *J. Microbiol. Biotechnol.* **24**, 648–660.
- Kim, T.W., Lee, J.H., Kim, S.E., Park, M.H., Chang, H.C., and Kim, H.Y.** 2009. Analysis of microbial communities in doenjang, a Korean fermented soybean paste, using nested PCR-denaturing gradient gel electrophoresis. *Int. J. Food Microbiol.* **131**, 265–271.
- Leclercq, R.** 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin. Infect. Dis.* **34**, 482–492.
- Lee, J.H., Kim, T.W., Lee, H., Chang, H.C., and Kim, H.Y.** 2010. Determination of microbial diversity in meju, fermented cooked soya beans, using nested PCR-denaturing gradient gel electrophoresis. *Lett. Appl. Microbiol.* **51**, 388–394.
- Murata, M., Ohno, S., Kumano, M., Yamane, K., and Ohki, R.** 2003. Multidrug resistant phenotype of *Bacillus subtilis* spontaneous mutants isolated in the presence of puromycin and lincomycin. *Can. J. Microbiol.* **49**, 71–77.
- Tatusov, R.L., Koonin, E.V., and Lipman, D.J.** 1997. A genomic perspective on protein families. *Science* **278**, 631–637.