Note (Genome Announcement)

Complete genome sequence of *Lactobacillus plantarum* JBE245 isolated from Meju

Jun Heo and Tai-Boong Uhm*

Department of Biological Sciences, Chonbuk National University, Jeonju 54896, Republic of Korea

메주에서 분리한 Lactobacillus plantarum JBE245 균주의 유전체 서열 분석

허 준·엄태붕* 전북대학교 자연과학대학 생명과학과

(Received September 20, 2017; Accepted November 1, 2017)

Lactobacillus plantarum is widely found in fermented foods and has various phenotypic and genetic characteristics to adapt to the environment. Here we report the complete annotated genome sequence of the *L. plantarum* strain JBE245 (= KCCM43243) isolated for malolactic fermentation of apple juice. The genome comprises a single circular 3,262,611 bp chromosome with 2907 coding regions, 45 pseudogenes, and 91 RNA genes. The genome contains 4 malate dehydrogenase genes, 3 malate permease genes and various types of plantaricin-synthesizing genes. These genetic traits meet the selection criteria of the strains that should prevent the spoilage of apple juice during fermentation and efficiently convert malate to lactic acid.

Keywords: Lactobacillus plantarum, fermented foods, genome sequence, lactic acid bacteria, Meju

Lactobacillus is a dominant genus of the lactic acid bacteria (LAB) that ferment carbohydrates to produce lactic acid as the major product (Bautista-Gallego *et al.*, 2013). Among them, *L. plantarum* has been found in various environments such as vegetables, meat, dairy products, and gastro-intestinal tracts (Di Cagno *et al.*, 2010; Siezen *et al.*, 2010). Due to the various phenotypic and genotypic characteristics of *L. plantarum*, this

species has been used in a variety of applications in the food fermentation and probiotics industries (Huys *et al.*, 2006). For this purpose, many of the genome analysis of these strains has been performed to understand what useful genetic traits *L. plantarum* with industrial usefulness has. In the previous work, we screened *L. plantarum* strains from various traditional foods for use as fermentation starters and finally isolated a starter strain JBE245 from fermented soybean lumps. This strain actively converted malate in the apple juice to lactate through malolactic fermentation (Heo *et al.*, 2016). Here, we report the complete genome sequence of *L. plantarum* JBE245 (= KCCM43243) and describe its genomic traits.

The genomic DNA was extracted from the stationary phase cells using a Wizard genomic DNA isolation kit (Promega). The whole genome of JBE245 was constructed *de novo* using Pacific Biosciences (PacBio) sequencing data. Sequencing data were assembled with PacBio SMRT analysis using the HGAP2 protocol (Pacific Biosciences; Chin *et al.*, 2013). Resulting contigs were scaffolded using GS Assembler (Roche Diagnostics). The annotation of each CDS was made through National Center for Biotechnology Information prokaryotic genome Annotation Pipeline (Tatusova *et al.*, 2016).

The genome statistics are provided in Table 1. The complete genome of JBE245 was composed of a single circular chro-

^{*}For correspondence. E-mail: tbuhm@chonbuk.ac.kr; Tel.: +82-63-270-3439; Fax: +82-63-270-3362

Table 1. Genome statistics of L. plantarum JBE245

Attribute	Values
Genome size (bp)	3,262,611
DNA coding (bp)	2,752,548
G + C content (%)	44.48
No. of contigs	1
Coding genes (CDS)	2,907
Pseudo genes	45
RNA genes	91

mosome and did not contain any plasmid DNA. The 3,262,611 bp genome with a G + C content of 44.48% contains 2,907 coding regions (CDS), 45 pseudogenes and 91 RNA genes (16 rRNA genes, 71 tRNA genes, and 4 non-coding RNA genes) based on NCBI's Prokaryotic Genome Annotation Pipeline (PGAP). The pseudogenes make stop codon in the middle of nucleotides sequences that encode proteins. Comparative analysis with other genomes of L. plantarum species indicates that 9 knock-out genes are discovered only in the genome of the JBE245. These regions can affect genetic traits and be used as biomarkers. Average nucleotide identity value (ANI) between the two strains JBE245 and RA-3 (accession No. GCA 001010175) is 99.65 to 99.71%, which exhibits the highest identity among the L. plantarum species registered in the NCBI database. The genome contains 4 malate dehydrogenase genes and 3 malate transporter genes. Especially, the malate dehydrogenases contain three types of enzyme classified as (S)-malate: NAD⁺ oxidoreductase (EC. 1.1.1.37), a (S)-malate: NAD⁺ oxidoreductase (decarboxylating) (EC. 1.1.1.38), and a (S)malate: NADP⁺ oxidoreductase (decarboxylating) (EC. 1.1.1.40), indicating a synergy effect in the malolactic fermentation.

A gene cluster for the synthesis of bacteriocins was also identified, including 3 plastaricin biosynthesis genes, 2 regulatory genes, 7 immunity genes, and 3 transport genes. These genetic traits meet the selection criteria of the strains that should prevent the spoilage of apple juice during fermentation and efficiently convert malate to lactic acid.

By analyzing the complete genome of *L. plantarum* JBE245 as well as comparative genomic studies with other species, we discovered the genetic traits in the genome and identified genes involved in malolactic fermentation of apple juice. Further studies of expression levels of the genes during fermentation by RNA sequencing will help to guide the biotechnological application of this strain.

Nucleotide sequence accession number

This whole genome sequence was deposited in GenBank under accession number CP014780.

적 요

발효 식품에서 널리 발견되는 Lactobacillus plantarum은 환경에 적응하기 위한 다양한 표현형 및 유전적 특성을 가지 고 있다. 우리는 사과 주스의 malolactic 발효를 위해 선발한 L. plantarum JBE245 (= KCCM43243) 유전체의 전체 염기서열 과 주석을 보고한다. 유전체는 2907개의 암호화 된 영역, 45개 의 위유전자, 91개의 RNA 유전자를 포함한 단일 원형 염색체 로 구성되었으며 그 크기는 3,262,611 bp였다. 이 유전체는 4 개의 malate dehydrogenase 및 3개의 malate permease 유전자 들과 함께 다양한 종류의 plantaricins 합성 유전자들을 포함하 고 있었다. 이러한 유전적 특성은 발효 동안 사과 주스의 부패 방지와 사과산(malate)의 젖산(lactate)으로의 효율적 전환이 요구되는 균주의 선발 기준과 잘 부합되었다.

Acknowledgements

This study was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ00999004)", Rural Development Administration, Republic of Korea.

References

- Bautista-Gallego, J., Arroyo-López, F.N., Rantsiou, K., Jiménez-Díaz, R., Garrido-Femández, A., and Cocolin, L. 2013. Screening of lactic acid bacteria isolated from fermented table olives with probiotic potential. *Food Res. Int.* 50, 135–142.
- Chin, C.S., Alexander, D.H., Marks, P., Klammer, A.A., Drake, J., Heiner, C., Clum, A., Copeland A., Huddleston, J., Eichler E., et al. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat. Methods* 10, 563– 569.
- Di Cagno, R., Minervini, G., Sgarbi, E., Lazzi, C., Bernini, V.,

Neviani, E., and Gobbetti, M. 2010. Comparison of phenotypic (Biolog System) and genotypic (random amplified polymorphic DNA-polymerase chain reaction, RAPD-PCR, and amplified fragment length polymorphism, AFLP) methods for typing *Lactobacillus plantarum* isolates from raw vegetables and fruits. *Int. J. Food Microbiol.* **143**, 246–253.

- Heo, J., Park H.S., and Uhm, T.B. 2016. Production of fermented apple juice using *Lactobacillus plantarum* JBE245 isolated from Korean traditional Meju. *Korean J. Food Sci. Technol.* 48, 445– 453.
- Huys, G., Vancanneyt, M., D'Haene, K., Vankerckhoven, V., Goossens,H., and Swings, J. 2006. Accuracy of species identity of

commercial bacterial cultures intended for probiotic or nutritional use. *Res. Microbiol.* **157**, 803–810.

- Siezen, R.J., Tzeneva, V.A., Castioni, A., Wels, M., Phan, H.T., Rademaker, J.L., Marjo, J.C., Starrenburg, M.J.C., Kleerebezem, M., Molenaar, D., et al. 2010. Phenotypic and genomic diversity of *Lactobacillus plantarum* strains isolated from various environmental niches. *Environ. Microbiol.* 12, 758–773.
- Tatusova, T., DiCuccio, M., Badretdin, A., Chetvernin, V., Nawrocki,
 E.P., Zaslavsky, L., Lomsadze, A., Pruitt, K.D., Borodovsky,
 M., and Ostell, J. 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res.* 44, 6614–6624.