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Genomic Insights of *Weissella jogaejeotgali* FOL01^T Reveals Its Food Fermentation Ability and Human Gut Adaptive Potential for Probiotic Applications in Food Industries

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Although the genus *Leuconostoc*, generally found in various fermented foods, has often been suggested to be a novel probiotic for food fermentation and health promotion, the strains in this genus showed low acid tolerance and low osmotic stress resistance activities, which are required for survival during food fermentation events. Recently, a novel species of *Weissella*, *W. jogaejeotgali* FOL01^T (= KCCM 43128 = JCM 30580), was isolated from Korean fermented clams. To determine the genomic features of this new species, its genome was completely sequenced and analyzed. The genome consists of a circular chromosome of 2,114,163 bp of DNA with a G+C content of 38.8%, and the plasmid pFOL01 consists of 35,382 bp of DNA with a G+C content of 39.1%. The genome analysis showed its potential for use in food fermentation and osmotic stress resistance abilities for processing in food industries. In addition, this strain was predicted to have acid tolerance and adhesion to the mucosal layer for survival and colonization in the gut. Subsequent experiments substantiated these abilities, suggesting that *W. jogaejeotgali* may have probiotic potential and a high survival rate during food fermentation. Therefore, it may be suitable as a novel probiotic strain for various applications in food industries.

Keywords: *Weissella jogaejeotgali*, lactic acid bacteria, probiotics, high-salt fermented food

In addition to various lactic acid bacteria (LAB), the genus *Leuconostoc*, belonging to the family Leuconostocaceae, has often been suggested as a novel probiotic because it is often found in various fermented foods, and its probiotic effects have been reported [1–3]. However, the genus *Leuconostoc* showed low acid tolerance and low osmotic stress resistance activities, which are required for survival during food fermentation. Owing to these weaknesses, the number of *Leuconostoc* generally decreases during food fermentation [4, 5]. However, the number of *Weissella* increases in this stage. The genus *Weissella* also belongs to the family Leuconostocaceae, and has been frequently found in various fermented foods, suggesting it is a lactic acid bacterial genus with Generally Recognized as Safe status [6]. Therefore, its strains may be better candidates as a novel probiotic than *Leuconostoc* strains.

In 1993, the genus *Weissella*, belonging to the LAB, was isolated and characterized [7]. Species in this genus have beneficial health effects, such as bacteriocin production (Weissellicin 110, Y, M, and L) and inhibition of adherence of *Helicobacter pylori* and biofilm formation of pathogens [8–11]. Owing to their wide distribution in various fermented foods and their putative probiotic traits, *Weissella* species are considered as a starter culture and potential probiotics for human health. Recently, a novel species, *W. jogaejeotgali* FOL01^T, was isolated from a traditional Korean high-salt fermented food, jogae-jeotgal [12]. In this study, the genome sequence of FOL01^T was entirely sequenced, and various probiotic properties were suggested at the genome level.

To determine the genetic basis for its probiotic potential for applications in food industries, the genome sequence of FOL01^T was completely sequenced and analyzed with various bioinformatic tools. The whole genome was sequenced using the PacBio RS II single-molecule real-time (SMRT) sequencing platform. The qualified sequence reads were assembled using the RS HGAP Assembly 2 in SMRT analysis ver. 2.3 [13]. The coding sequences were predicted using a combination of GeneMarkS [14] and Glimmer3 [15], and their functions were predicted and annotated using the Rapid Annotation using a Subsystem Technology (RAST) server [16], InterProScan5 [17], and KEGG database [18]. The tRNA and rRNA genes were predicted using the RAST server. Tolerance to osmotic and pH stresses (0 to 14% final NaCl concentration and pH 3 to 7) was monitored during incubation of strain FOL01^T in MRS broth for 48 h at 37°C. A test for adhesion ability to mucin was conducted according to a previously developed protocol [19].

Table 1. Genome features of *Weissella jogaejeotgali* FOL01^T.

	Chromosome	Plasmid
Genome size (bp)	2,114,163	35,382
GC content (%)	38.8	39.1
Total ORFs	2,016	51
Genes with predicted function	1,583	35
rRNA	25	-
tRNA	75	-

The genome features of FOL01^T are listed in Table 1, and its genome map is illustrated in Fig. 1. The complete genome of FOL01^T consists of a 2,114,163 bp circular chromosome (G+C content of 38.8%) and a 35,382 bp single plasmid (G+C content of 39.1%). The chromosome contains 2,016 open reading frames (ORFs), 75 tRNA genes, and 25 rRNA genes. In addition, the plasmid contains 51 ORFs.

The carbohydrate fermentation abilities of FOL01^T were previously reported, suggesting that this strain may be suitable for food fermentation [12]. In addition, oligopeptide

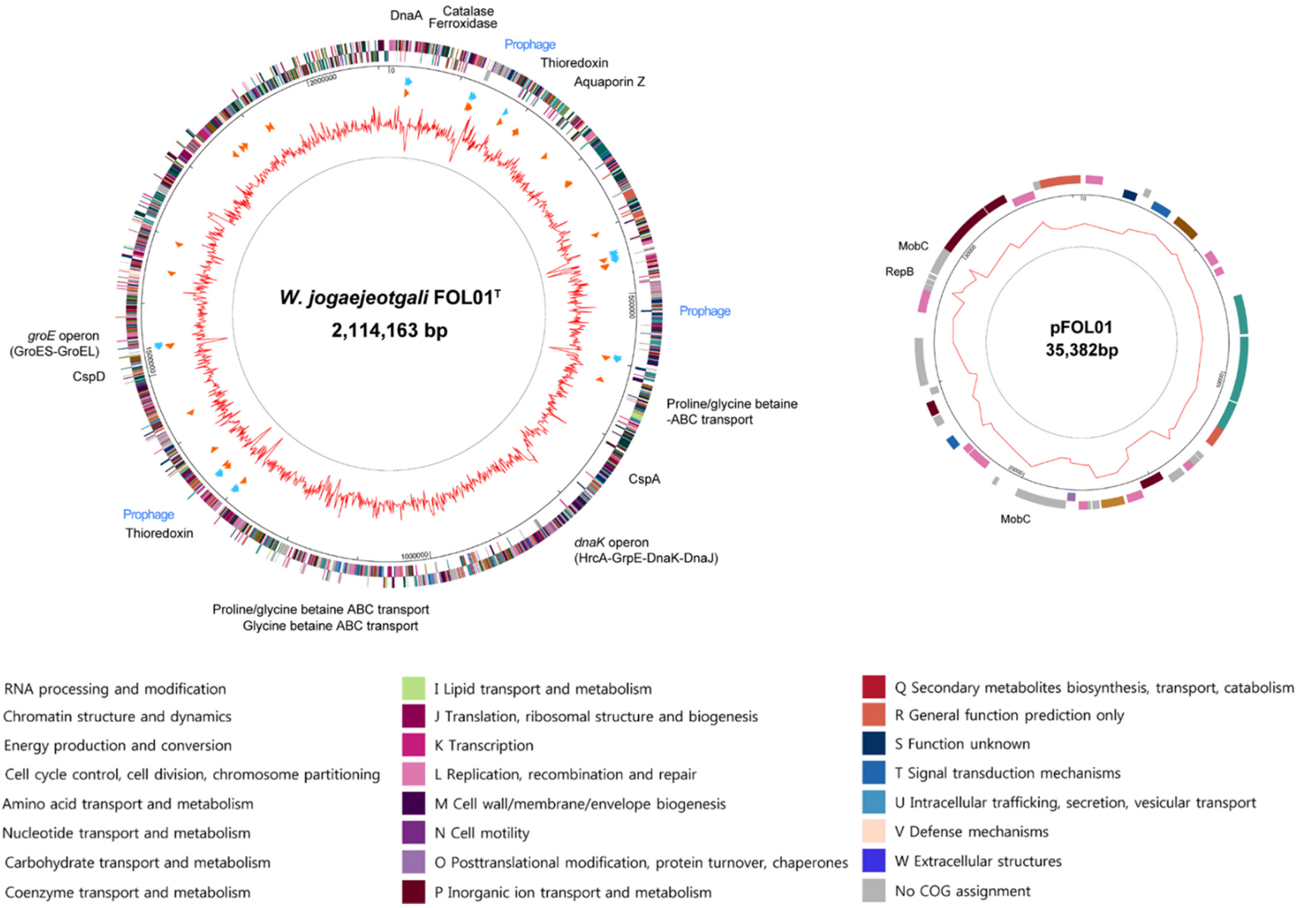

Fig. 1. Circular genome map of *Weisella jogaejeotgali* strain FOL01^T.

The outer circle indicates the location of all annotated ORFs, and the inner circle with red peaks indicates the GC content. Between these circles, sky blue arrows indicate the rRNA operons, and orange arrows indicate the tRNAs. Functional gene clusters are labeled around the outer circle as follows: bacteriophages in blue, and other functional gene clusters in black. All annotated ORFs are colored according to the COG assignments.

ABC transport systems (encoded by *oppABCDE*) and aminopeptidases were detected, suggesting that this strain has successfully adapted to the oligopeptide-rich environment of jogae-jeotgal. These suppositions were supported by the presence of only 10 amino acid metabolic pathway genes (aspartate, arginine, alanine, threonine, methionine, glutamate, glutamine, cysteine, serine, and tryptophan) in the genome. Tolerances to growth-inhibitory conditions such as high salinity and low pH are important characteristics of a probiotic strain [20]. The genome analysis revealed that strain FOL01^T has aquaporin Z (FOL01_0217 and 1864) and glycine betaine ABC transporters (FOL01_0579, 1058, 1076, 1144, and 1188) [21], suggesting a high salinity-resistant ability, which would be important for survival in highly salted jogae-jeotgal [22]. An osmotic stress test using different concentrations of sodium chloride revealed that this strain grew well at an up to 7% NaCl concentration, but had a 70% growth reduction in 8% NaCl concentration (Fig. 2A), indicating higher osmotic stress resistance than various *Leuconostoc* strains, which showed 70% reduction in 5.5% NaCl [1]. This genome also has acid tolerance-associated genes encoding F1F0 ATP synthase (FOL01_1762-1769), cation transport ATPase (FOL01_0375), cystathionine gamma-lyase (FOL01_0208), cystathionine gamma-synthase (FOL01_1979), and cystathionine beta-lyase (FOL01_1980) [23], probably related to survival during passage through the stomach and small intestine. A subsequent acid tolerance test showed that this strain was fully grown up to pH 5, but showed 60% growth reduction at pH 4.5 (Fig. 2B), indicating higher acid tolerance activity than various *Leuoconostoc* strains demonstrating 80% growth reduction at pH 5 [1]. In addition, FbpA fibronectin binding proteins (FOL01_0040 and 1208) were detected, suggesting that FOL01^T may have not only intestinal colonization ability but inhibition activity toward colonization of pathogen [24]. A comparative mucin adhesion test showed that this strain has the highest binding activity among closely related *Weissella* strains [12] (Fig. 3). Furthermore, genome annotation and the PathogenFinder analysis [25] showed that FOL01^T is non-pathogenic toward animals and humans.

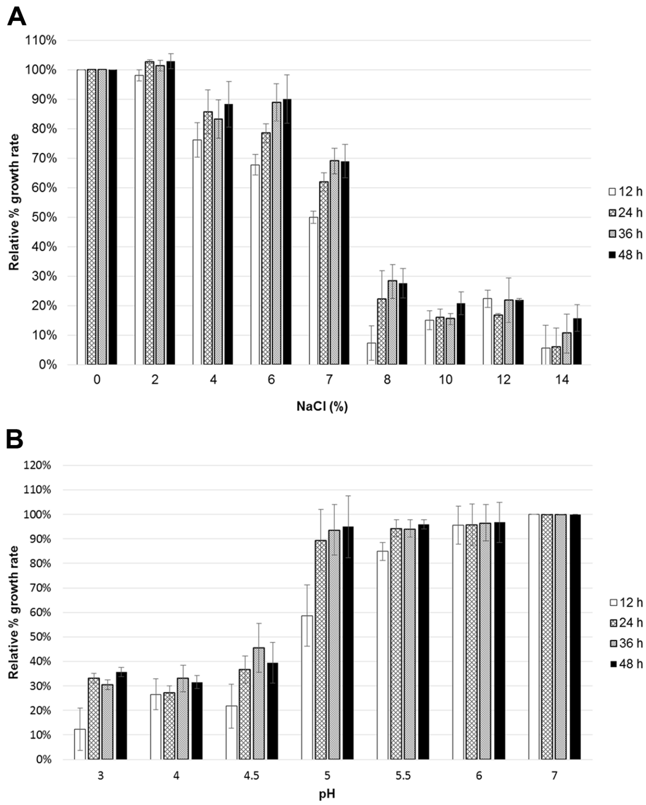

Fig. 2. Tolerance tests of *Weisella jogaejeotgali* strain FOL01^T under various osmotic and pH conditions.

(**A**) Osmotic stress test with different NaCl concentrations from 0 to 14%. (**B**) Acid tolerance test with different pH conditions from 3 to 7. All experiments were performed in triplicates, and standard deviations are indicated with error bars.

These distinct features of strain FOL01^T indicate that it may have food fermentation and probiotic potentials for various food applications in industries. This is the first report of genome information of a novel species, *W. jogaejeotgali* FOL01^T, providing its genomic properties for further understanding its roles in food applications. Based on this study, the genus *Weissella* may be a better candidate as a novel probiotic for various food applications than the genus *Leuconostoc*, even though they belong to the same family.

The complete genome sequence and annotation data of *W. jogaejeotgali* FOL01^T have been deposited to the GenBank Data Library under the accession numbers CP014332 (chromosome) and CP014333 (plasmid). The strain is available in public culture collections (KCCM 43128 and JCM 30580).

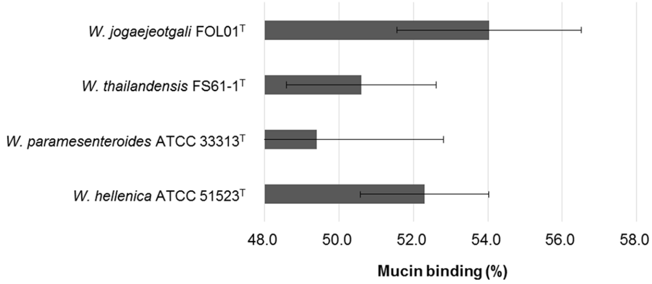

Fig. 3. Mucin adhesion test of *Weisella jogaejeotgali* strain FOL01^T and other three closely related *Weissella* species (*W. thailandensis*, *W. paramesenteroides*, and *W. hellenica*).

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