Lactobacillus Plantarum LRCC5314 Includes a Gene for a Substance that Stimulates the Secretion of Serotonin

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Research Article

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Abstract

Background: As the functions of probiotics within the same species may not be shared, it is important to analyze the genetic characteristics of strains to determine their safety and usefulness before industrial applications.

Results: The complete genome of *Lactobacillus plantarum* LRCC5314 isolated from kimchi was acquired through PacBio sequencing to confirm probiotic functions and genetic characteristics. Phylogenetic and comparative genomic analyses were performed between *L. plantarum* LRCC5314 and *L. plantarum* ATCC 14917^T, and the results showed that two strains were highly similar. However, there were some genetic differences. The characteristics of carbon source metabolism were also determined based on the KEGG database and physiological properties. *L. plantarum* LRCC5314 could metabolize hexoses through homofermentation, which produces only lactic acid. According to gene annotation, *L. plantarum* LRCC5314 could encode almost complete biosynthetic pathway to produce tryptophan, which can be used as a precursor of serotonin. The serotonin ELISA test using Caco-2 and HT-22 cells treated with a supernatant of the bacterial culture showed that more serotonin was produced by treated cells than by untreated cells.

Conclusion: *L. plantarum* LRCC5314 could provide a source for serotonin production, and *L. plantarum* LRCC5314 could be used as a functional probiotic for stress regulation.

Background

The genus *Lactobacillus* is a type of lactic acid bacteria (LAB) with diverse species. These rod-shaped Gram-positive bacteria are found in various environments, such as silage, fermented foods, fecal samples [1–3], and the human body, including the gastrointestinal tract and oral cavity [4, 5]. Species of the genus *Lactobacillus* are widely used to manufacture dairy products, such as cheese, yogurt, and kefir starter [6–8]. In addition, some strains of lactobacilli, including *L. rhamnosus, L. plantarum, L. casei*, and *L. raffinolactis*, not only have fermentation functions but also have beneficial effects on human health as consumable probiotics.

According to the Food and Agriculture Organization/World Health Organization (FAO/WHO), probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [9]. Probiotics modulate the gut environment by balancing the microflora. Several LAB, such as lactobacilli or lactococci, are recognized as probiotics, as they have different functions, such as lowering cholesterol or controlling immune reactions [10, 11]. Over recent decades, *L. plantarum* and *L. rhamnosus* have been investigated for their beneficial effects on diabetes, obesity, liver disorder, and stress [12, 13]. These days, along with medical supplies, probiotics are used to improve and prevent health problems.

However, comparative studies among probiotic strains have shown that the features of probiotics and their corresponding health benefits are strain-specific. These benefits may not be generalizable and may

not be shared among strains, even among those of the same species [14]. For this reason, the genomic characterization of probiotics is important for the study of beneficial functions or mechanisms. Moreover, genome-based analysis is required to investigate the stability and safety of probiotics for industrial applications. Probiotics also exert negative effects through undisclosed genes. Virulence or antibiotic resistance genes can be transferred to other bacteria from probiotics via horizontal gene transfer [15]. Whole-genome analysis of probiotic strains can prevent potential risks by predicting them and thus increasing utilization efficiency.

L. plantarum LRCC5314 was isolated from kimchi as a probiotic candidate. In this study, we investigated the whole genome of *L. plantarum* LRCC5314 to characterize the strain via comparative, genome content, and metabolic pathway analyses. In addition, we identified the functional factors of *L. plantarum* LRCC5314 through gene annotation and provided the molecular biological evidence for its practical use as a probiotic strain.

Materials And Methods

Phylogenetic and physiological characteristics of *L. plantarum* LRCC5314

The genomic DNA of *L. plantarum* LRCC5314 was extracted using the genomic G-spin DNA extraction kit for bacteria (Intron, Seongnam, Korea). The sequence of 16s rRNA was amplified by polymerase chain reaction (PCR) with the universal primers 8F and 1525R [33] and purified using the Accuprep PCR purification kit (Bioneer, Seoul, Korea). The purified gene was sequenced directly, and the sequence similarity levels among closely related strains were calculated with EzBioCloud (https://www.ezbiocloud.net/identify) [34]. The phylogenetic distance between *L. plantarum* LRCC5314 and related strains of the genus *Lactobacillus* was calculated using CLUSTAL-X 2.1 software [35]. The phylogenetic trees were constructed using the neighbor-joining, maximum-parsimony, and maxi-mum-likelihood methods with MEGA 7 software [35-38]. To determine the physiological characteristics of *L. plantarum* LRCC5314, API 50CH, API 20E, and API ZYM (Biomerieux, Marcy-l'Étoile, France) tests were performed according to the manufacturer's instructions.

Whole-genome sequencing and annotation

The whole genome of *L. plantarum* LRCC5314 was sequenced using the PacBio RS platform with the 20kb SMRTbell TPK library kit. The sequences were assembled de novo with RS HGAP Assembly (version 3.0) in PacBio SMRT Analysis software (version 2.3). Genomic annotation was performed using the RAST server (version 2.0) [39], and the National Center for Biotechnology Information (NCBI)'s Prokaryotic Genomes Annotation Pipeline 4.1 [40] was used to combine the results. WebMGA on-line tools (version 2.2.15; http://weizhong-lab.ucsd.edu/webMGA/) [41] were used to group protein functions with the COG database. Antimicrobial resistance genes were identified using the program ResFinder (version 4.1; https://cge.cbs.dtu.dk/services/ResFinder/) [42]. The carbon source metabolic pathway was constructed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [43]. The sequences

generated in this study were deposited in the DDBJ/EMBL/GenBank International Nucleotide Sequence Database under the BioProject ID PRJNA716786.

Metabolic pathway analysis of carbon sources

Based on KEGG pathway and protein BLAST [44] analyses, the carbon metabolic pathways of *L. plantarum* LRCC5314 were constructed. The pyruvate, pentose phosphate, sucrose, lactose, and galactose metabolism pathways of the strain were mapped according to the KEGG pathway. To construct a pathway, only mapped genes were used. Protein BLAST was used to reconfirm the functions of the characteristic genes.

Comparative genomic analysis of *L. plantarum* LRCC5314

The whole-genome sequence of *L. plantarum* ATCC 14917^T (GCF_000143745) was obtained from NCBI GenBank for comparative genomic analysis with *L. plantarum* LRCC5314. The digital DNA-DNA hybridization (dDDH) value with *L. plantarum* ATCC 14917T was calculated using the Genome-to-Genome Distance Calculator (GGDC; version 2.1; http://ggdc.dsmz.de/ggdc.php) [45]. OrthoANI values between *L. plantarum* LRCC5314 and related strains in the genus Lactobacillus were calculated using Orthologous Average Nucleotide Identity Tool (OAT) software [46]. A circular map to compare the genome sequence between *L. plantarum* LRCC5314 and *L. plantarum* ATCC 14917^T was created using Blast Ring Image Generator (BRIG) software [47]. CRISPRFinder [48] was used to analyze clustered regularly interspaced palindromic repeats (CRISPRs).

Production of bacterial supernatant

L. plantarum LRCC5314 was cultured at 30°C under aerobic conditions in De Man, Rogosa and Sharpe (MRS) broth (BD Difco, Franklin Lakes, NJ, USA) overnight. After reaching an OD of 1.0 at 600 nm, the cultured broth was centrifuged for 10 min at 8,000 × g. The supernatant was filtered with a 0.22 μm filter after harvesting and kept at −20°C until use.

Cell culture

The cell lines Caco-2 and HT-22 were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and Merck (Darmstadt, Germany), respectively. Caco-2 cells were cultured in Minimum Essential Medium (MEM; Gibco, Waltham, MA, USA) supplemented with 10% fetal bovine serum and 1% penicillin. HT-22 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Lonza, Basel, Switzerland) supplemented with 10% fetal bovine serum and 1% penicillin. The cell lines were incubated at 37°C with 5% CO_2.

Measurement of serotonin release

The cultured Caco-2 and HT-22 cells were seeded in 24-well plates (1 × 105 cells/well) and incubated until sub-confluent. Then, the cells were treated with the bacterial super-natant and incubated for 1 h at 37°C.

The supernatants of the treated wells were collected after incubation and centrifuged at 1,000 × g for 3 min. The serotonin content of the cell supernatants was quantified with the Serotonin High Sensitive ELISA kit (IBL, Hamburg, Germany) according to the manufacturer's instructions.

Statistical analysis

Multiple comparisons were performed by t-test and GraphPad Prism (v.6.0.1) (Graph Pad Software Inc., La Jolla, CA, USA). All data are presented as the mean ± standard error of the mean (SEM), and p values < 0.05 were considered significant.

Results

Phylogenetic and phenotypic features of the isolated LAB strain

The 16S rRNA gene of L. plantarum LRCC5314 was obtained and compared with available reference sequences from the GenBank database and EzBioCloud (http://www.ezbiocloud.net/eztaxon). Phylogenetic analysis based on the 16S rRNA gene showed that *L. plantarum* LRCC5314 was clustered with the *L. plantarum* species (Fig. 1). *L. plantarum* LRCC5314 was the most closely related to *L. plantarum* ATCC 14917^T with a similarity of 100%, followed by *L. pentosus* (99.9%), *L. paraplantarum* (99.7%), and *L. daowaiensis* (99.1%). The results showed that the isolated LRCC5314 strain was a strain of L. plantarum.

According to the API test, *L. plantarum* LRCC5314 could utilize glycerol, L-arabinose, D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, D-sorbitol, N-acetylglucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-melibiose, D-saccharose, D-trehalose, D-melezitose, gentiobiose, D-furanose, potassium gluconate, and potassium 5-ketogluconate. *L. plantarum* LRCC5314 could produce acetoin from sodium pyruvate and was negative for NO_2 and H_2S production. The API ZYM test showed that *L. plantarum* LRCC5314 was positive for the activities of alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naph-thol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, and N-acetyl-β-glucosaminidase.

Genomic characterization

The whole-genome sequence of *L. plantarum* LRCC5314 consisted of 3,249,803 bp. The chromosome contained 16 rRNAs (5S rRNA, 6; 16S rRNA, 5; 23S rRNA, 5), 67 tRNAs, and 3,031 protein-coding sequences (CDSs). The G+C content of *L. plantarum* LRCC5314 was 44.5 mol% (Table 1). WebMGA online tools were used to analyze *L. plantarum* LRCC5314 protein functions annotated in the COG database. The annotated proteins were related to the categories of translation, ribosomal structure and biogenesis (J; 147 genes), transcription (K; 259 genes), replication, recombination, and repair (L; 146 genes), cell cycle control, cell division, and chromosome partitioning (D; 23 genes), defense mechanisms (V; 62 genes), signal trans-duction mechanisms (T; 72 genes), cell wall/membrane/envelope biogenesis (M; 174 genes), intracellular trafficking, secretion, and vesicular transport (U; 22 genes), post-translational

modification, protein turnover, and chaperones (O; 61 genes), energy pro-duction and conversion (C; 106 genes), carbohydrate transport and metabolism (G; 268 genes), amino acid transport and metabolism (E, 199 genes), nucleotide transport and metabolism (F; 86 genes), coenzyme transport and metabolism (H; 64 genes), lipid transport and metabolism (I; 55 genes), inorganic ion transport and metabolism (P; 128 genes), and secondary metabolite biosynthesis, transport, and catabolism (Q; 17 genes; Fig. S1).

RAST analysis annotated genes to assign them to the categories of amino acids and derivatives (15.8%), cofactors, vitamins, prosthetic groups, and pigments (9.5%), and nucleosides and nucleotides (8.0%). Genes in the amino acids and derivatives category included those related to “glutamine, glutamate, aspartate, asparagine, and ammonia assimilation” (19 genes), “histidine metabolism” (8 genes), “arginine, urea cycle, and polyamines” (3 genes), and “lysine, threonine, methionine, and cysteine” (82 genes). Genes in the cofactors, vitamins, prosthetic groups, and pigments category included those related to “biotin” (3 genes), “riboflavin, FMN, and FAD” (20 genes), “pyridoxine” (8 genes), “NAD and MADP” (9 genes), “folate and pterines” (43 genes), “lipoic acid” (3 genes), and “co-enzyme A”. Genes in the nucleosides and nucleotides category included those related to “pyrimidines” (25 genes), “purines” (47 genes), and “detoxification” (6 genes; Table S1).

Comparative genomic analysis

OrthoANI analysis between *L. plantarum* LRCC5314 and closely related *Lactobacillus* strains showed similarities in the range of 75.14–99.65%. *L. plantarum* LRCC5314 was the most closely related to *L. plantarum* ATCC 14917^T (99.65%), followed by *L. paraplantarum* DSM 10667^T (85.90%). *L. pentosus* DSM 2031^T, *L. herbarum* NBRC 10733^T, and *L. plajomi* NBRC 10733^T showed similarities of 79.80%, 77.18%, and 74.77%, respectively (Fig. 2A). The dDDH value with *L. plantarum* ATCC 14917^T was 97%. OrthoANI and dDDH values showed that the LRCC5314 strain belonged to the species *L. plantarum*. Furthermore, based on BLAST comparison, the whole-genome sequences of *L. plantarum* LRCC5314 and *L. plantarum* ATCC 14917^T showed high similarity (Fig. 2B). However, a circular comparison of the genomes of *L. plantarum* LRCC5314 and *L. plantarum* ATCC 14917^T showed differences in the GC content and genomic similarities between the two strains. Furthermore, *L. plantarum* LRCC5314 contained a CRISPR in its genome. According to CRISPRFinder, *L. plantarum* LRCC5314 had a CRISPR without Cas genes between 1,536,174 bp and 1,536,259 bp, whereas no confirmed CRISPRs were detected in *L. plantarum* ATCC 14917^T. These results demonstrated that the LRCC5314 strain belonged to the same species (*L. plantarum*) as the ATCC 14917^T strain but had genetic differences.

Carbon source metabolic pathway

The metabolic pathways of carbon sources related to the genes of *L. plantarum* LRCC5314 were combined and mapped in one synthesis pathway (Fig. 3). For carbo-hydrate metabolism, 228 genes of *L. plantarum* LRCC5314 were mapped to the KEGG pathway, and only the metabolic pathways of carbon sources with a positive result in the API 50CH test were combined. *L. plantarum* LRCC5314 could utilize various carbon sources, including glucose, sucrose, maltose, and lactose. According to the carbon source

metabolic pathway, *L. plantarum* LRCC5314 could produce pyruvate from glucose via glycolysis and could not produce alcohol. These findings revealed the homofermentative activity *L. plantarum* LRCC5314, which could metabolize hexoses via the Embden-Meyerhof-Parnas pathway.

Tryptophan metabolism and serotonin production

According to gene annotation, the *L. plantarum* LRCC5314 genome contained a complete biosynthetic pathway for producing tryptophan from chorismate (Fig. 4 and Table S2). Tryptophan is an essential amino acid for humans because it is not synthesized in the human body, and humans need to consume it. However, only 1% of the available tryptophan is used for protein synthesis, and 99% is used as a precursor of tryptamine, melatonin, and serotonin.

Tryptophan metabolism and serotonin production

To determine the stimulatory effects of *L. plantarum* LRCC5314 on 5-hydroxytryptamine (5-HT) production, Caco-2 and HT-22 cells were treated with the bacterial supernatant, and the content of 5-HT was measured with the ELISA method. Caco-2 cells were used as an in vitro model of the intestinal epithelial barrier. In the in vitro experiment with Caco-2 cells, 5-HT secretion was increased more than 1.5 times in the cell supernatant of treated Caco-2 cells compared with untreated cells (untreated versus treated Caco-2 cells: 7.2 ± 0.15 versus 12.8 ± 0.7 pg/mL, $p < 0.001$; Fig. 5A). The secretion of 5-HT from HT-22 cells treated with the bacterial supernatant was al-so increased with statistical significance (untreated versus treated HT-22 cells: 17.5 ± 1.12 versus 19.9 ± 0.03 pg/mL, $p < 0.05$; Fig. 5B). In comparison with untreated cells, Caco-2 and HT-22 cells treated with the *L. plantarum* LRCC 5314 supernatant showed a statistically significant increase in 5-HT secretion. These results suggest that a component of the bacterial supernatant stimulated 5-HT secretion.

Discussion

L. plantarum is one of the probiotics commonly found in various fermented dairy products or traditional fermented foods such as kimchi or sausage [3], and its safety and beneficial effects on human health have been established. This bacterium is a versatile species that can be isolated from various environments, including fermented vegetables [16]. OrthoANI analysis showed that *L. plantarum* LRCC5314 was the most closely related to *L. plantarum* ATCC 14917T with a similarity of 99.65%. The G+C content range of the *L. plantarum* species has been reported to be 44.37–44.71 mol% [17], and *L. plantarum* LRCC5314 had a numerical value coinciding with the average [18]. These results indicated the LRCC5314 strain belonged to *the L. plantarum* species.

According to the API test, the LRCC5314 strain showed a positive result for β-galactosidase. β-galactosidase, also known as lactase, is an enzyme that catalyzes lactose into glucose and galactose. People who lack this enzyme can suffer from lactose intolerance and may be unable to consume dairy products [19]. However, probiotic strains with β-galactosidase can support lactose digestion in the small intestine. Bile salts from the gallbladder stimulate the lactase activity of probiotics and mitigate lactose

intolerance symptoms [20]. *L. plantarum* LRCC5314 was positive for the activation of the β-galactosidase enzyme in the API test, suggesting that the strain can be used as a probiotic to alleviate lactose intolerance in the human intestine.

Hexoses are monosaccharides that contain six carbon atoms, such as mannose, glucose, and fructose. LAB can be classified as homofermentative and heterofermentative based on how they metabolize hexoses. Heterofermentative LAB metabolize hexoses via the acetyl-phosphate and phosphoketolase pathway, whereas homofermentative LAB metabolize hexoses via the Embden-Meyerhof-Parnas pathway [21]. *L. plantarum* species, including *L. plantarum* LRCC5314, are homofermentative LAB and metabolize hexoses to pyruvate, lactate, carbon dioxide, and ethanol through the Embden-Meyerhof-Parnas pathway. *L. plantarum* LRCC5314 could utilize D-galactose, D-glucose, D-fructose, and D-mannose according to the API 50CH test and could metabolize them via glycolysis, which was verified by in silico analysis.

The European Food Safety Authority (EFSA) recommends that bacterial strains with antibiotic resistance genes should not be used as probiotics for humans or as an additive to animal feeds [22]. The drug resistance genes of probiotic strains have been a focus of re-search because of their risk of transfer to other microbial species in the human intestine [23]. ResFinder was used to detect genes conferring antibiotic resistance in the *L. plantarum* LRCC5314 genome, and none were detected. The gene search results showed that *L. plantarum* LRCC5314 may be safe as a potential probiotic strain without risk of antibiotic gene transfer.

In our in vitro experiment, the bacterial supernatant promoted the secretion of serotonin in Caco-2 and HT-22 cells. 5-HT regulates the digestive process at several levels within the human gastrointestinal system. When foods enter the gastrointestinal tract, digestion is carried out through peristaltic waves, which are modulated by 5-HT [24]. The HT-22 cell line is an immortalized mouse hippocampal cell line and is used to identify the release of 5-HT at the brain level. The hippocampus is part of the limbic system and plays an important role in the conversion of short-term memories to long-term memories and emotional regulation [25]. Furthermore, hippocampal cells have the 5-HT receptor, the 5-HT transporter (5-HTT), and tryptophan hydroxylase (the rate-limiting enzyme in 5-HT pro-duction), which were discovered in these cells previously [26].

Serotonin, also known as 5-HT, is synthesized via a short pathway from L-tryptophan, in which tryptophan hydroxylase and aromatic L-amino acid decarboxylase are involved [27]. This monoamine neurotransmitter has multifaceted and complex biological functions. 5-HT has been reported to play functional roles in cognition, mood modulation, learning, memory, and various physiological processes [28]. Around 90% of 5-HT is found in the gastrointestinal tract, especially in enterochromaffin cells. 5-HT secreted from enterochromaffin cells acts in synergy with other digestive hormones to control intestinal motility. However, 1–2% of serotonin is produced in nerve cells, such as neurons with the 5-HT receptor. 5-HT produced at the end of neurons is released to the synapse and serves to transmit signals to other neurons [29]. This process is also known to be involved in the regulation of stress hormones such as

cortisol, and a deficiency in 5-HT can cause depression and bipolar disorder. Therefore, several antidepressants function to prevent the absorption of 5-HT [30].

However, 5-HT cannot directly cross the blood-brain barrier (BBB). For the biosynthesis of serotonin in the brain, a precursor needs to be transported into the BBB. Tryptophan enters the brain through a common transporter protein and competes with other neutral amino acids for transport [31]. The amount of tryptophan entering the brain through the transporter protein is proportional to the concentration of tryptophan in the plasma. Several studies have reported that tryptophan-enriched diets could increase 5-HT levels in the brain and decrease cortisol levels [32].

Conclusion

We analyzed the complete genome of *L. plantarum* LRCC5314 and shed light on its genomic features, carbon source metabolic pathway, and functional genes. Genomic analysis confirmed that *L. plantarum* LRCC5314 had the tryptophan biosynthetic gene, which could produce tryptophan, a precursor that can be used in 5-HT synthesis. Moreover, in vitro analysis demonstrated that *L. plantarum* LRCC5314 could stimulate 5-HT se-creation in intestinal epithelial cells and serotonergic neurons. These results suggest that *L. plantarum* LRCC5314 may be used as a beneficial probiotic to alleviate mental stress and possibly to improve mental health.

Abbreviations

LAB: lactic acid bacteria; FAO/WHO: Food and Agriculture Organization/World Health Organization; PCR: polymerase chain reaction; NCBI: National Center for Biotechnology Information; COG: Clusters of orthologous groups; KEGG: Kyoto Encyclopedia of Genes and Genomes; dDDH: digital DNA-DNA hybridization; BRIG: Blast Ring Image Generator; MRS: De Man, Rogosa and Sharpe medium; ATCC: American Type Culture Collection; DMEM: Dulbecco's Modified Eagle Medium; ELISA: Enzyme-linked immunosorbent assay.

Declarations

Availability of data and materials

The data set of this study have been deposited to DDBJ/EMBL/GenBank International Nucleotide Sequence Database under the BioProject ID PRJNA716786.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they do not have any competing interests.

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Authors' contributions

Conceptualization, WK.; conducted the experiments, JJ. and SY.; Analyzed data, JJ., SY. and JHK.; writing—original draft preparation, JJ. and SY.; writing—review and editing, JHK. and WK.; visualization, JHK.; supervision, WK.; project administration, WK.; funding acquisition, WK. All authors have read and agreed to the published version of the manuscript.

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Supplementary Information

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Tables

Table 1. The genomic characterization of *L. plantarum* LRCC5314 and *L. plantarum* ATCC 14917^T (ACGZ02000000) was performed. CRISPR loci were analyzed using CRISPRFinder.

	L. plantarum LRCC5314	*L. plantarum* ATCC 14917^T
Genome size (bp)	3,249,803	3,212,261
GC content (%)	44.5	44.6
Annotated genes	3,306	3,011
tRNAs	67	67
rRNAs	16	16
CRISPR loci	1	0

Figures

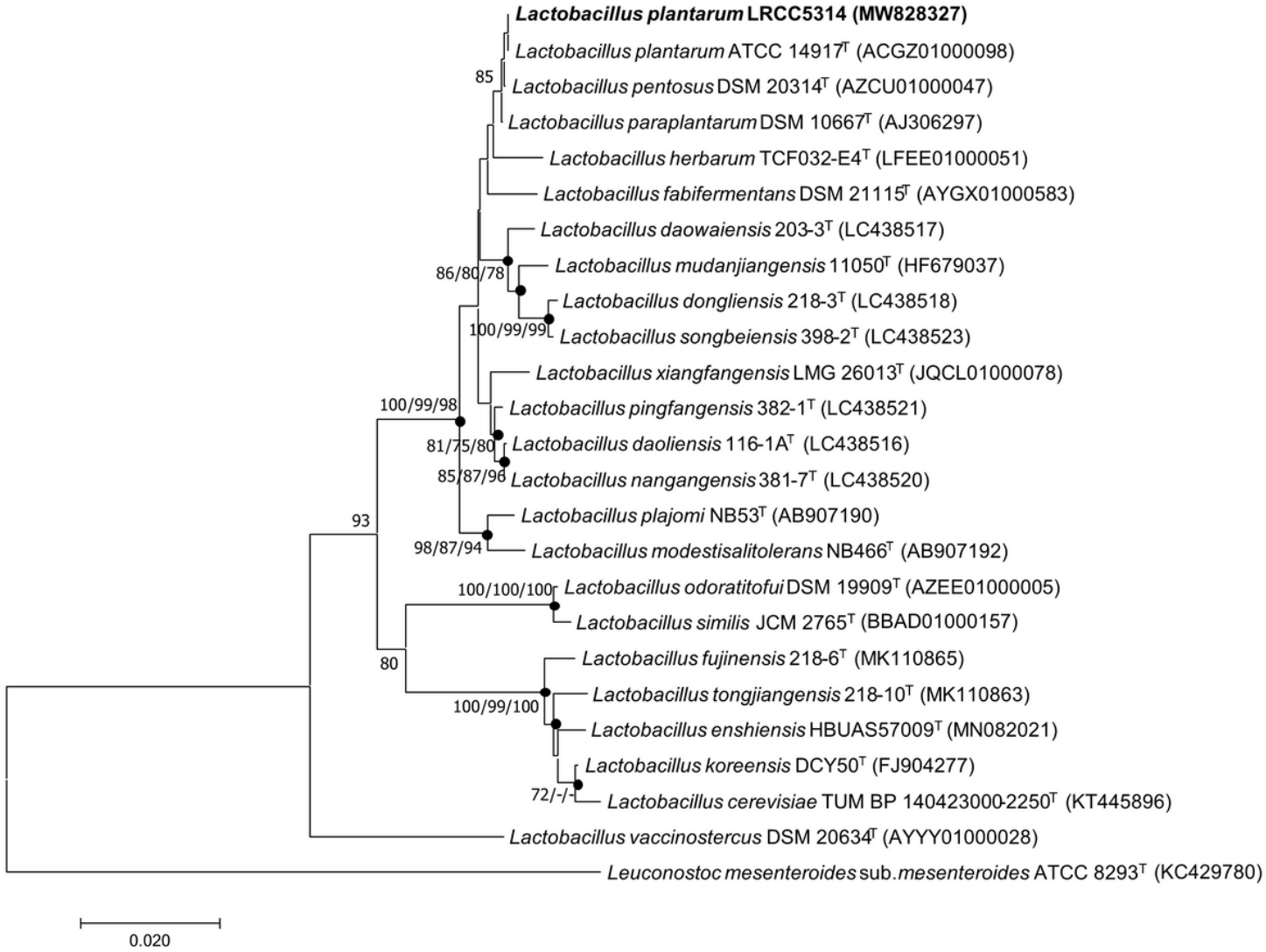

Figure 1

Phylogenetic tree constructed with the neighbor-joining method for L. plantarum LRCC5314 and related strains of the genus Lactobacillus. The filled circle indicates that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. The numbers at the nodes indicate the percentage of bootstrapping based on 1,000 resampling; only values > 70% are given. Bar, 0.02 substitutions per nucleotide position. Leuconostoc mesenteroides subsp. mesenteroides ATCC 8293T (KC429780) was used as an outgroup organism.

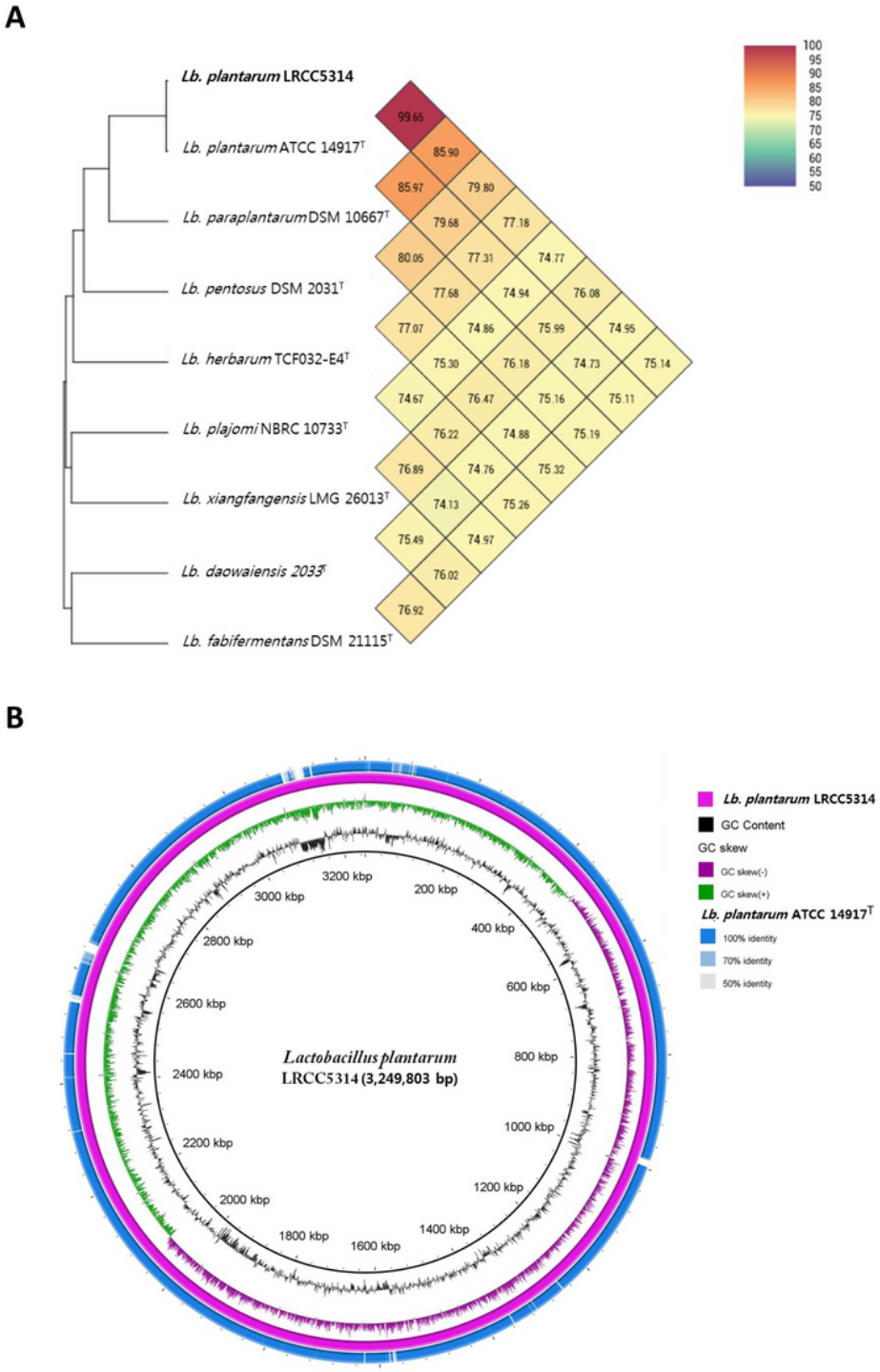

Figure 2

Comparative genomic analysis of L. plantarum LRCC5314 and Lactobacillus strains. (A) OrthoANI values between L. plantarum LRCC5314 and closely related Lactobacillus strains (L. plantarum ATCC 14917T, L. paraplantarum DSM 10667T, L. pentosus DSM 2031T, L. herbarum TCF032-E4T, L. plajomi NBRC 10733T, L. xiangfangensis LMG 26013T, L. daowaiensis 2033T, and L. fabifermantans DSM 21115T). (B) Circular comparison of the genomes of L. plantarum LRCC5314 and L. plantarum ATCC 14917T. From outside to inside, the first circle shows the genome of L. plantarum ATCC 14917T, the second circle shows the genome of L. plantarum LRCC5314, the third circle shows the GC skew, and the fourth circle shows the GC content.

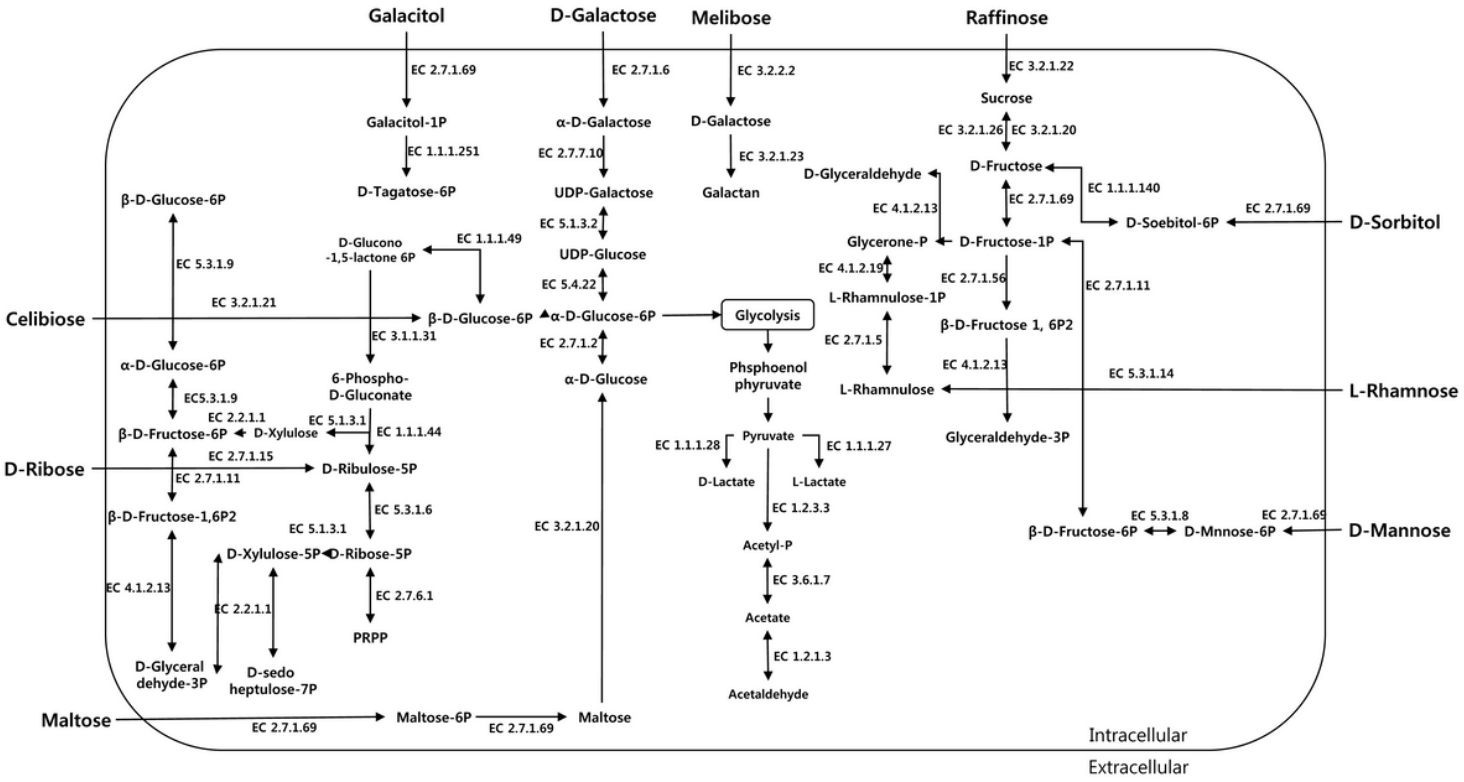

Figure 3

Carbon source metabolic pathway of L. plantarum LRCC5314. Various carbon compounds are involved in the overall metabolic pathway of L. plantarum LRCC5314.

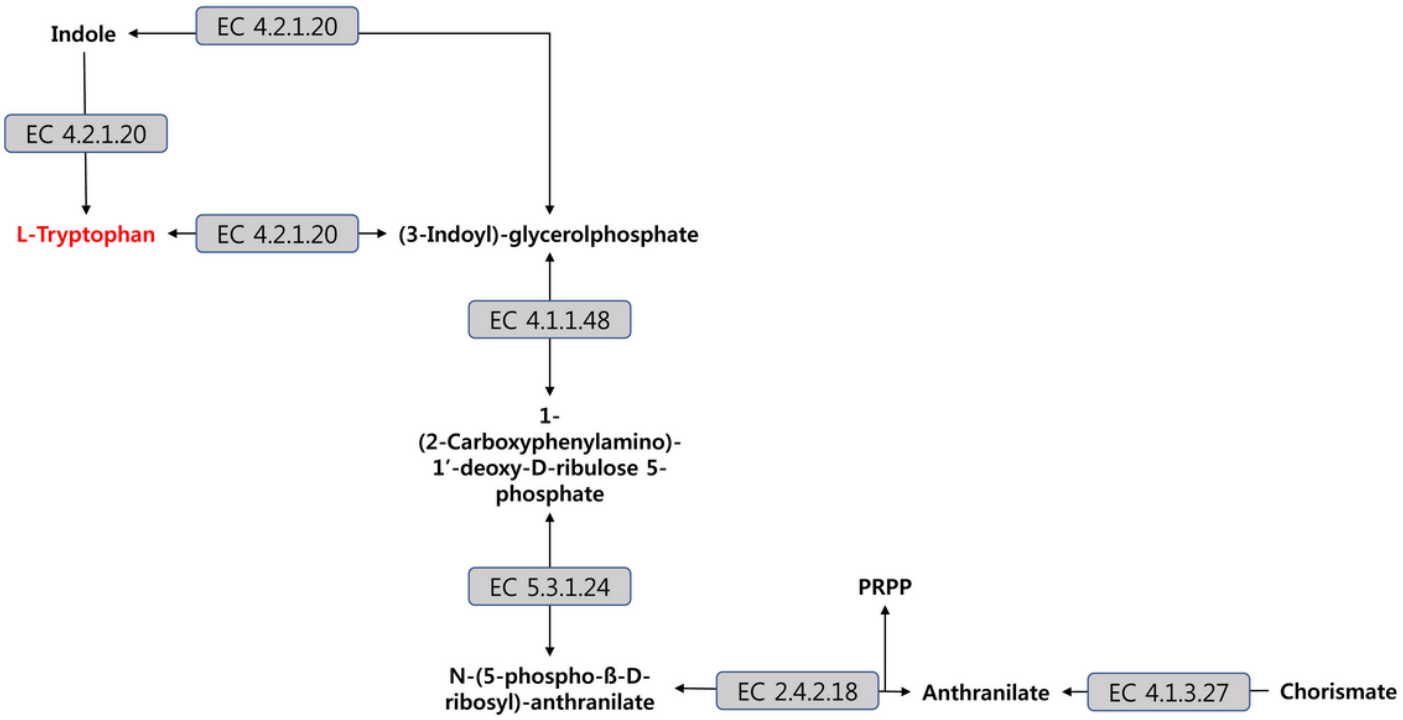

Figure 4

Tryptophan biosynthesis in L. plantarum LRCC5314. The tryptophan biosynthetic pathway and related enzymes are shown. Grey boxes indicate enzymes in the tryptophan bio-synthetic pathway present in L. plantarum LRCC5314.

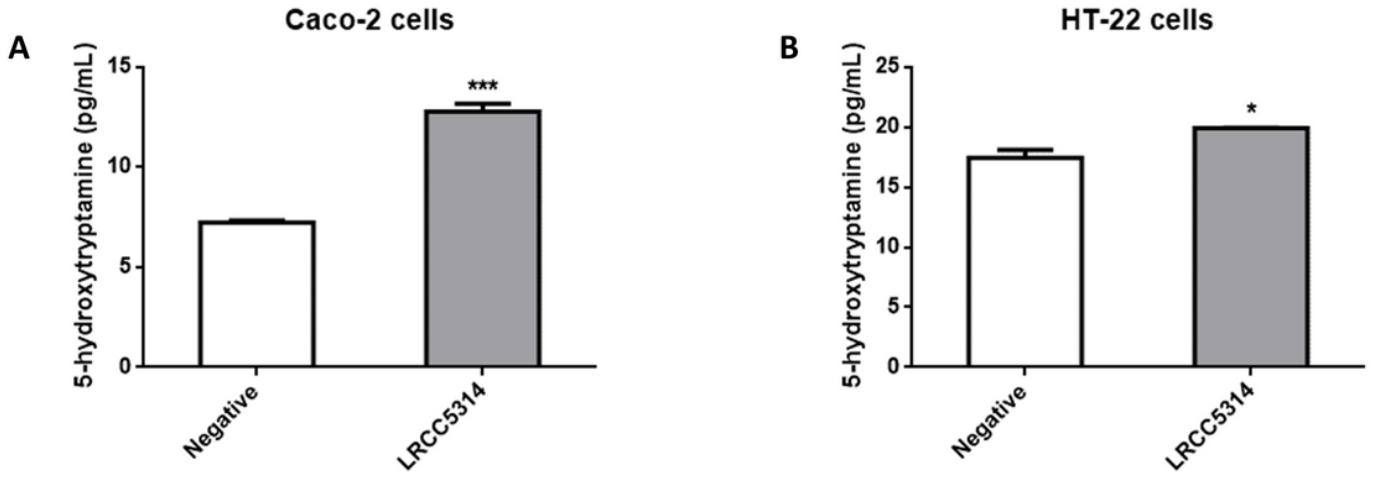

Figure 5

Concentration of 5-hydroxytryptamine secreted by the Caco-2 and HT-22 cell lines. The concentration of 5-hydroxytryptamine (5-HT) from (A) Caco-2 cells and (B) HT-22 cells was measured after treatment with

the culture supernatant of L. plantarum LRCC5314. *p < 0.05; ***p < 0.001.

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