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The effects of next generation probiotics on metabolic dysfunction-associated steatotic liver disease: a parallel, double-blind, randomized, placebo-controlled trial

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Abstract

Background Metabolic dysfunction-associated steatotic liver disease (MASLD) is associated with dysbiosis of the gut microbiota. We evaluated the effect of next generation probiotics (*Lactobacillus delbrueckii subsp. Lactis* [LL001], *L. helveticus* [LH001], and *Pediococcus pentosaceus* KID7 [PPKID7]) on liver function parameters and stool microbiome in patients with MASLD.

Methods We conducted a double-blind parallel trial of 110 patients diagnosed with MASLD. Participants were randomly assigned to four groups given three probiotics (3 capsules [9×10^9 CFU]/day, $n=85$) or placebo ($n=25$) alongside sylimarin for 8 weeks. Clinical characteristics, serum samples, and stool samples for 16 S rRNA gene sequencing were collected at the start and end point of the study. The primary endpoint was improvement in liver function.

Results In the probiotic group, LL001 treatment improved alanine transaminase (87.3 ± 8.2 to 71.1 ± 6.0 U/L, $P=0.01$) and aspartate transaminase levels (64.9 ± 4.9 to 50.0 ± 3.5 U/L, $P<0.01$), LH001 group showed body weight reduction (78.4 ± 3.0 to 77.2 ± 2.8 kg, $P=0.01$), and PPKID7 reduced cholesterol levels (186.1 ± 7.0 to 178.0 ± 7.9 mmol/L, $P=0.03$). Probiotics treatment decreased the abundance of *Proteobacteria* and increased the abundance of *Ruminococcaceae* and *Lachnospiraceae* in the LL001 group. In the pre- and post-comparison of probiotic treatment at the level of the top 20 genera, a tendency was observed to decrease the genera *Haemohlius* and Ruminococcus_g2 while increasing the genus *Bifidobacterium*.

Conclusion Eight weeks of probiotics supplementation was associated with changes in the stool microbiome and improvements in the blood biochemical parameters of MASLD.

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Registration number of clinical trial NCT04555434.

Keywords Probiotics, Gut, Microbiota, Metabolic dysfunction-associated steatotic liver disease, Improvement

Introduction

Metabolic dysfunction-associated fatty liver disease applies to adults exhibiting hepatic steatosis identified through imaging techniques, blood biomarkers, or liver histology [1]. This diagnosis is made in individuals who are overweight or obese, or who have type 2 diabetes mellitus, or at least two other metabolic risk abnormalities. Subsequently, in June 2023, a multisociety Delphi consensus statement was issued on a revised nomenclature for fatty liver diseases. This statement introduced the term metabolic dysfunction-associated steatotic liver disease (MASLD), effectively replacing the previous nonalcoholic fatty liver disease [2]. Representative etiologies of MASLD are known to include insulin resistance, lipid toxicity due to excessive fat accumulation, inflammatory response, and endoplasmic reticulum stress [3–5].

The global rise in obesity has contributed to the increased prevalence of MASLD [6]. MASLD affects approximately 30% of the global adult population, with its prevalence rising from 22% to 37% between 1991 and 2019 [7]. This increase aligns with the growing rates of obesity and related conditions worldwide. The more severe manifestation of MASLD, known as metabolic dysfunction-associated steatohepatitis (MASH), is histologically characterized by lobular inflammation and hepatocyte ballooning and is linked to a higher risk of fibrosis progression [8, 9]. Among individuals diagnosed with MASLD who do not meet criteria for a liver biopsy, the prevalence of MASH is approximately 7% [10]. MASLD is one of the most common causes of chronic liver disease and liver-related death and serves as a risk factor for extrahepatic diseases such as diabetes and cardiovascular disease [11–13]. As a result, the burden of social and economic costs caused by the disease is increasing [14, 15].

Recently, interest in the role of the gut microbiome in the pathogenesis and treatment of liver diseases has increased, and the disease linkage caused by intestinal bacterial imbalance has been identified [16, 17]. Animal studies have demonstrated potential causal roles of the gut microbiota in MASLD [18, 19]. Human studies have described microbiome alterations in healthy individuals and patients with MASLD and have found several consistent specific taxa that differentiate between healthy individuals and patients with MASLD and advanced liver disease [20, 21]. Despite unknown mechanisms and lack of validation, therapeutic strategies utilizing the gut microbiome have the potential to provide beneficial effects in patients with MASLD.

Probiotics are live microorganisms that, when administered in adequate amounts, provide health benefits to the host [22, 23]. In our previous animal studies, *Lactobacillus* and *Pediococcus* supplementation improved MASLD by modulating gut microbiota and inflammation [24, 25]. Additional experiments with candidate strains have shown promising results in the prevention of MASLD progression (Fig. S1). Several randomized controlled trials using ultrasonography and MASLD diagnostic markers of short duration have suggested that administration of probiotics may have a positive effect on hepatic steatosis and insulin resistance in patients [26, 27]. However, none of the previous studies reported the therapeutic effect of probiotics on the microbiome in patients with MASLD. In this clinical trial, we aimed to investigate whether administering probiotics for 8 weeks could effectively improve blood biochemical parameters and induce alterations in the gut microbiota among patients diagnosed with MASLD.

Materials and methods

Patients

A total of 120 participants with MASLD were recruited from the Hallym University Chuncheon Sacred Heart Hospital. In this double-blind placebo-controlled clinical trial, 120 subjects were divided into four groups using a randomization procedure, including three intervention groups and one placebo group and an allocation ratio of 1:1:1:1. group 1 (LL001) received probiotic capsules (*L. lactis* CKDB001: 9.0×10^9 CFU), group 2 (LH001) received *L. helveticus*, group 3 (PPKID7) received *P. pentosaceus* KID7, and group 4 (Placebo) received probiotic capsules (microcrystalline cellulose powder).

Participants visited two weeks prior to the clinical trial and were screened using the inclusion and exclusion criteria (Fig. 1A). The participants, with a mean age of 47.0 ± 13.6 years and comprising 61% men, were randomly assigned to four groups alongside Sylimarin (Legalon®, Bukwang, Seoul) for 8 weeks. A total of 110 out of 120 participants (91.7%) completed the clinical trial (67 men and 43 women). The probiotic groups received capsules (9×10^9 CFU per day)(Fig. 1B). Table S1 shows the pre-trial baseline clinical characteristics of each randomized group. At baseline, there were several differences in the main clinical and biochemical parameters between the probiotics and placebo groups (Table 1). Participants in all groups had BMI (kg/m^2) measurements above the normal range at

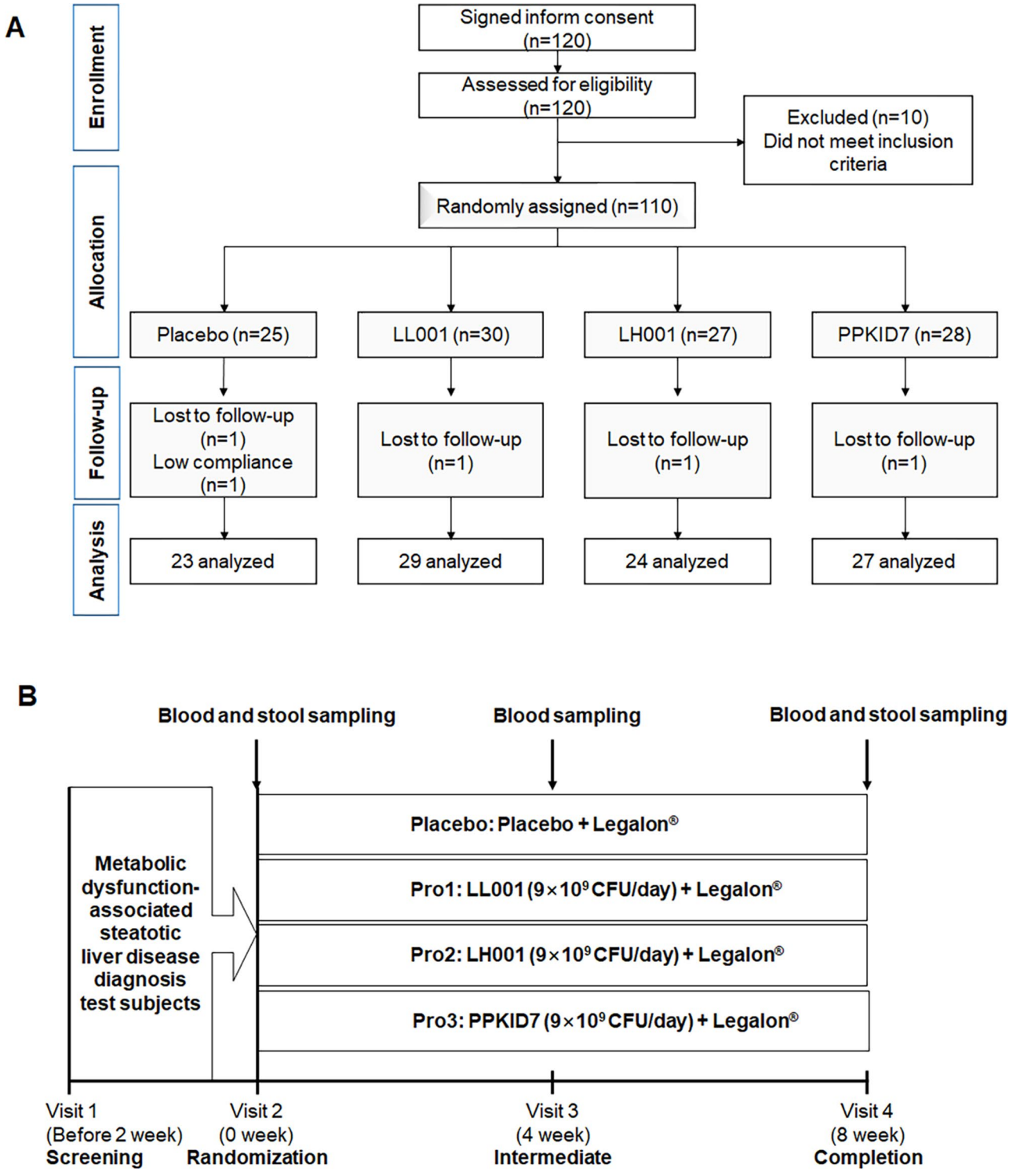


Fig. 1 Study design. (**A**) Overall process of trial. (**B**) Flow of study. Schematic of clinical trial. Visit 1 (V1): participants who consented to the clinical trial are assessed for their suitability according to the selection/exclusion criteria. Visit 2 (V2): Randomly assigned after a 2-week run-in period. Test drug and placebo intervention, blood and stool sampling. Visit 3 (V3): 4th week of intake. Blood sampling. Visit 4 (V4): Termination of clinical trial and analyze results. Blood and stool sampling

Table 1 Parameters measured in all groups at baseline and after 8 weeks of treatment

Variables	Placebo ($n=23$)		LL001 ($n=29$)		LH001 ($n=24$)		PPKID7 ($n=27$)	
	Baseline	End of Study	Baseline	End of Study	Baseline	End of Study	Baseline	End of Study
Weight (*kg*)	87.0 ± 3.4	86.6 ± 3.6	77.7 ± 2.9	77.4 ± 2.9	78.4 ± 3.0	77.2 ± 2.8*	79.8 ± 3.1	78.6 ± 3.2
BMI, kg/m^2	29.9 ± 0.9	29.7 ± 0.9	28.8 ± 0.7	28.7 ± 0.8	28.2 ± 0.7	27.8 ± 0.7*	29.2 ± 0.9	28.7 ± 1.0
ALT, *U/L*	72.3 ± 5.1	81.9 ± 6.4	87.3 ± 8.2	71.1 ± 6.0*	77.0 ± 6.5	80.4 ± 7.7	77.7 ± 5.8	70.4 ± 5.2
AST, *U/L*	47.0 ± 2.6	44.3 ± 2.9	64.9 ± 4.9	50.0 ± 3.5**	54.4 ± 4.0	48.3 ± 3.9	53.7 ± 3.9	49.2 ± 5.1
Chol, *mmol/L*	187.2 ± 6.3	186.6 ± 8.1	178.4 ± 6.2	175.0 ± 7.3	196.8 ± 8.5	193.6 ± 8.8	186.1 ± 7.0	178.0 ± 7.9*
Alb, *g/Dl*	4.7 ± 0.1	4.6 ± 0.0	4.6 ± 0.1	4.5 ± 0.1	4.6 ± 0.0	4.6 ± 0.0	4.7 ± 0.0	4.7 ± 0.0
γGT, *U/L*	50.2 ± 5.8	50.4 ± 8.4	80.9 ± 11.0	76.7 ± 9.0	61.5 ± 8.3	56.9 ± 5.9	62.4 ± 7.5	55.1 ± 6.2
PT, *s*	11.6 ± 0.1	11.9 ± 0.2	12.0 ± 0.2	11.7 ± 0.2**	11.5 ± 0.2	11.3 ± 0.1	11.5 ± 0.1	11.5 ± 0.1
INR	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0*	1.1 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.0

Data are expressed as mean + SEM. Significant difference from baseline value. *$P<0.05$, **$P<0.01$. Nonparametric two-tailed Wilcoxon matched-pairs signed rank tests were performed to verify changes from baseline in each group. ALT, Alanine transaminase; AST, Aspartate transaminase; Chol, cholesterol; Alb, albumin; γGT, γ-Glutamyltransferase; PT, prothrombin time; INR, international normalized ratio

baseline (Fig. S2). After 8 weeks, body weight and BMI decreased in all groups compared to baseline. Among the tested drugs, only the LH001 group showed a significant decrease in body weight and BMI compared to baseline.

Study design and participants

This clinical study was designed as a proof-of-concept, double-blind placebo-control clinical trial (NCT04555434). After a 2-week run-in period, groups were randomly assigned in the order of registration, placebo and test drug, and blood and stool samples were collected. The placebo and test drug were instructed to be administered orally with sufficient water after meals three times a day, and it was conducted for 8 weeks. Four weeks after the start, a revisit was performed, blood was collected once more, and blood and stool samples were collected at the end of the test at the 8th week (Fig. 1B). The primary endpoint was improvement in liver function and the secondary endpoints were change in stool microbiotas and stress analysis.

A total of 120 patients were screened during the study period. Eligible participants were randomly assigned to the four groups. Among these patients, 10 were excluded before randomization, seven were lost to follow-up, and 103 patients (23 in the placebo group, 29 in the LL001 group, 24 in the LH001 group, and 27 in the PPKID7 group) completed the study with follow up.

MASLD was diagnosed based on the definition described in the consensus statement on the new fatty liver disease nomenclature [28, 29]. Cardiometabolic criteria including body mass index, fasting glucose, medication history, blood pressure, or cholesterol level, were used to diagnose MASLD. The normal control group did not meet cardiometabolic criteria. Patients with elevated liver enzyme levels [AST or ALT ≥ 50 IU/L] or hepatitis on liver pathology were included in the MASLD group.

Exclusion criteria were: consumption of probiotics (lactic acid bacteria, etc.), prebiotics (dietary fiber, fructooligosaccharide, etc.), synbiotics, and fermented milk in the month before the study; continuously taking antibiotics within the last 2 months or take antibiotics during the study period; continuously consumed medicines or health functional foods that affect liver function within the last 1 month; participation in other clinical trials within the last 1 month (however, this does not apply to medical device clinical trials); alcoholic liver disease, hereditary metabolic disease, autoimmune hepatitis; systemic inflammatory disease or immune disease; hepatocellular carcinoma or a history of malignant tumor diagnosis within the last 5 years; uncontrolled cardiopulmonary disease; other serious systemic disorders of the heart, lungs, blood, and endocrine system; pregnant or lactating women; hypersensitivity to the test drug/placebo or components contained in the test drug/placebo or experienced severe allergic reactions.

This project followed the ethics of the 1975 Helsinki Declaration, as reflected by a prior approval by the Chuncheon Sacred Heart Hospital Institutional Review Board for human research (2019-08-005-011). Informed consent was obtained from all the participants. All authors had access to the study data and reviewed and approved the final manuscript.

Sample size

This clinical study was designed as a proof-of-concept study by the principal investigator; therefore, sample size calculation was not performed during initiation; However, it was planned to enroll at least 30 patients in each group.

Randomization and blinding

Random allocation sequences were generated via an online randomization tool employing permuted block sizes of variable length. Study products (probiotics and

placebo) were indistinguishably packaged and coded with unique identification numbers before distribution to participating sites. Upon enrollment, research nurses assigned each participant the next sequential study code according to the randomization list. Allocation information was concealed from participants, investigators, and clinical staff until the database was locked and analyses were completed.

Intervention

The dosage of probiotics was three capsules per day for 8 weeks. Placebos of the same shape and size were manufactured by Chong Kun Dang Pharmaceutical Corporation (Seoul, Korea). All patients were treated with Legalon® (silymarin; Bukwang Pharm Co. Ltd, Seoul, Korea) capsules (450 mg/day).

Baseline studies included family history, diet pattern, alcohol history, abdominal ultrasound and computed tomography scan, X-ray, electrocardiography, complete blood count, electrolytes, liver function test, viral markers, and Child–Pugh score. Blood analyses were performed using standard method. Serum biochemical parameters included AST, ALT, cholesterol, albumin, γGT, bilirubin, blood urea nitrogen, creatinine, international normalized ratio (INR), α-fetoprotein, carcinoembryonic antigen, prothrombin time (PT), blood glucose, and total cholesterol. The levels of hepatitis A, B, and C and other viral markers were evaluated. Antinuclear antibody, antimitochondrial antibody, and antismooth muscle antibody tests were also performed.

Preparation of probiotics

Lactobacillus delbrueckii subsp *lactis* CKDB001 (1), *Lactobacillus helveticus* CKDB001 and *Pediococcus pentosaceus* KID7 (2) are lactic acid-producing bacteria that have been isolated from sour milk, homemade ricotta cheese and fermented finger millet (*Eleusine coracana*) gruel respectively. The stocks of each strain were prepared by mixing the cultured broth with an equivalent 20% skim milk solution and then stored at -80 °C until use. The stocks of each strain were spread on MRS agar plate and incubated under the facultative anaerobic condition at 37 °C for 48 h. After incubation, single colony of each strain was inoculated in MRS broth and cultured under the facultative anaerobic condition at 37 °C for 24 h. After seed culture, cultured broth of each strain was inoculated in optimized medium and fermented at 37 °C for 16 h and pH was maintained constantly (pH 5.5 or 6.0 ± 0.5). After fermentation, cells were harvested and concentrated by centrifugation. Concentrated cells were lyophilized for 72 h and ground and packaged in polyethylene and aluminum bags. Packaged strains were stored at 4 °C before dispatch in CKDBiO Corp., Ltd. (Ansan-si, South Korea). Probiotics and placebo capsules were provided by Mediogen (Chungju-si, South Korea). Probiotic capsules were composed of each strain and microcrystalline cellulose powder, named LL001 (*L. lactis* CKDB001), LH001 (*L. helveticus* CKDB001), or PPKID7 (*P. pentosaceus* KID7). The texture, color, and odor of microcrystalline cellulose powder were identical to that of the probiotic and placebo vehicle. To check the quality of four products, contamination analysis of coliform group bacteria, heavy metals, residual pesticides, and nutritional analysis were performed and approved by the Korea Advanced Food Research Institute of the Korea Food Industry Association (Uiwang-si, South Korea).

Stool microbiome analysis

Sequencing was carried out according to the manufacturer's instructions at Chunlab, Inc. (Seoul, Republic of Korea) with the Illumina MiSeq. Microbiome taxonomic profiling was conducted with the EZBioCloud platform (ChunLab Inc., Republic of Korea) using the database version PKSSU4.0.

Human feces were stored at -20 °C as soon as the patient received 2–3 g of feces using the kit (stool paper and stool box) and moved to -80 °C within 1 day. Genomic DNA for metagenomic sequencing was extracted with a QIAamp stool kit (Qiagen, Hilden, Germany), and the library was prepared with a NEBNext Ultra II FS DNA Library Prep Kit for Illumina (New England BioLabs, Ipswish, MA, USA) according to the manufacturer's directions. The quantification of libraries was checked using a Qubit dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA, USA) and confirmed by quantitative polymerase chain reaction (qPCR) with a KAPA SYBR FAST qPCR Master Mix kit (Kapa Biosystems, Wilmington, MA, USA). The quality and product size were assessed with an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA, USA) using a DNA 7500 chip. The library pool was sequenced with 250-bp paired-end reads on the Illumina MiSeq systems(Illumina, San Diego, CA, USA) according to the manufacturer's instructions.

The analysis was performed following our previous reference [30]. In brief, DNA was extracted with a QIAamp stool kit, and amplification of the V3-V4 region of the bacterial 16 S rRNA gene was conducted using barcoded fusion primers. The forward fusion primer contained the p5 adapter, i5 index, and gene-specific primer 341 F (5′-AATGATACG GCGACCACCGAGATCTACAC-XXXXXXXX-TC GTCGGCAGCGTCAGATGTGTATAAGAGACA G-CCTACGGGNGGCWGCAG3′; underlining indicates the target region primer and X indicates the

barcode region), and the reverse fusion primer contained the p7 adapter, i7 index, and gene-specific primer 805R (5′-CAAGCAGAAGACGGCATACGAGATXXXXXXXXGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GACTACHVGGGTATCTAATCC-3′), which included sequencing adapters and dual-index barcodes of the Nextera XT kit (Illumina, San Diego, CA, USA). The amplification was performed in the C1000 touch thermal cycler PCR system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) with the following conditions: initial denaturation of 3 min at 95 °C; followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s and final extension at 72 °C for 5 min. Each amplified PCR product was confirmed with 1% agarose gel electrophoresis and visualized on a Gel Doc XR + imaging system (Bio-Rad Laboratories, Inc., USA). The amplified products were purified and size-selected by Agencourt AMPure XP beads (Beckman Coulter, Chaska, MN, USA).

Raw reads were quality-filtered (Trimmomatic v0.32, Q25), merged (VSEARCH v2.13.4), and processed through chimera removal (UCHIME) and non-specific amplicon filtering (HMMER v3.2.1). OTUs were clustered at 97% similarity, and taxonomy was assigned using the EzBioCloud 16 S rRNA database (version PKSSU4.0).

Raw demultiplexed reads per sample ranged from 25,178 to 128,050 (median 45,943), and Post-QC read depths ranged from 18,175 to 81,537 (median 30,147), with rarefaction curves plateauing around 18–20k reads/sample. A rarefaction depth of 18,000 reads/sample was applied to retain all samples while ensuring diversity saturation.

Statistical analysis

For statistical analysis of baseline demographics and clinical data, a full analysis set was used. Data are reported as mean ± standard error of the mean (SEM). For all parameters, and within each group, the intervention effect was calculated by subtracting the value obtained at visit 2 from the value obtained at visit 4 weeks for each participant. This value was named the 'mean difference'. The mean difference from the placebo was calculated by subtracting the mean difference calculated for the placebo group from the mean difference calculated for the probiotic test group. The abundance of *Lactobacillus* and *Bifidobacterium* genera was log-transformed due to outliers, and the changes were statistically analyzed. Unpaired t-tests or non-parametric Mann–Whitney U-tests were used to assess the significance of differences between the mean differences of all groups. Paired t-tests or non-parametric, two-tailed, matched-pairs Wilcoxon signed-rank tests were performed to identify differences between visit 2 and visit 4 within each group. In the microbiome analysis, Benjamini and Hochberg false discovery rate (FDR) was performed to correct for multiple testing. One-way analysis of variance (ANOVA) or Kruskal–Wallis tests was used to compare baseline and differential values across all groups. Account for baseline variability between treatment groups and calculated adjusted mean differences (± standard error) and corresponding 95% confidence intervals for each treatment group compared to the placebo group by an analysis of covariance (ANCOVA) model (Supplementary Table S2).

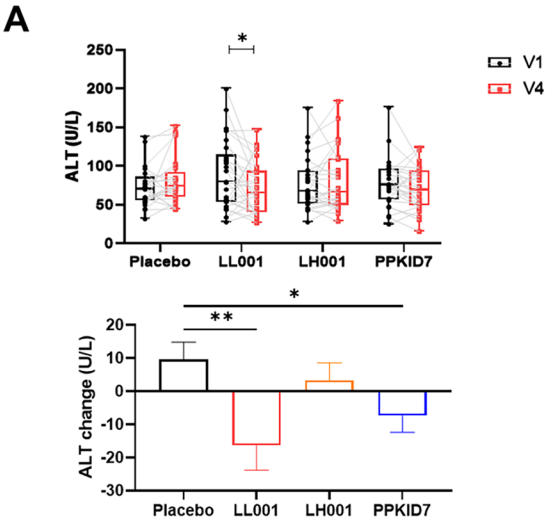
This study investigated the gut–liver axis using an orally administered probiotic formulation. To evaluate the efficacy of the administered probiotic, we applied the intention-to-treat approach. The per-protocol analysis was not adopted because it could introduce bias and reduce the accuracy of the efficacy assessment. Instead, the ITT method was chosen to minimize potential confounding variables and preserve the randomization principle. Subjects without available pre- and post-treatment data were excluded from the final analysis.

Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software, Boston, MA, USA) and R version 4.3.1 (R Foundation for Statistical Computing) with the emmeans package (v1.11.0). ANCOVA models were fitted using the base lm function in R, and adjusted means and pairwise contrasts were obtained using emmeans. A two-sided $p < 0.05$ was considered statistically significant.

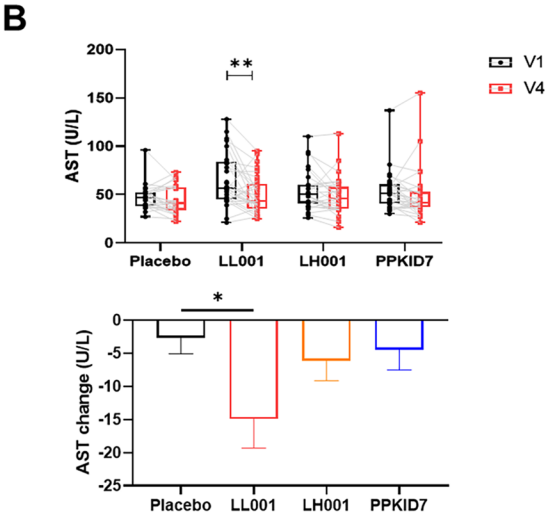
Results

Primary outcome: biochemistry indices by probiotics treatment

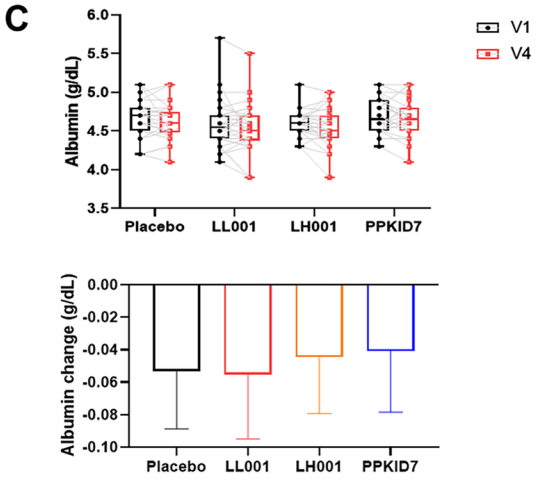
Participants in all groups had aspartate transferase (AST) and alanine transferase (ALT) levels above the normal range at baseline. After 8 weeks, the placebo group showed no significant difference in ALT levels (9.64 ± 5.21 U/L), but there was an increase. In contrast, participants in the group who received LL001 showed a significant decrease ($P = 0.01$, -16.23 ± 7.56 U/L) in ALT levels compared to baseline after 8 weeks, and this effect was significant between the LL001 and placebo groups ($P = 0.002$; Fig. 2A). The PPKID7 group was also significantly different ($P = 0.02$) from the placebo group, but the difference (-7.36 ± 5.05 U/L) from baseline within the group was only decreased and not significant. After 8 weeks, the placebo group identified no significant difference (-2.64 ± 2.43 U/L) of AST levels (Fig. 2B). In contrast, participants in the three groups who received test drugs showed a decrease of AST levels (LL001, -14.90 ± 4.42 U/L; LH001, -6.09 ± 3.04 U/L; PPKID7, -4.44 ± 3.08 U/L) compared



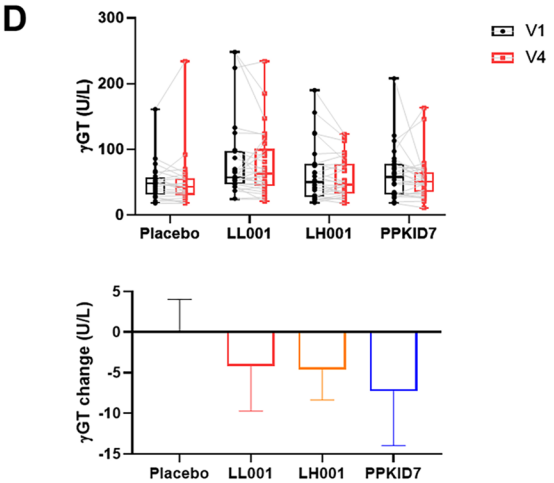
	Placebo	LL001	LH001	PPKID7
Mean difference	9.64±5.21	-16.23±7.56	3.37±5.27	-7.36±5.05
Adj. mean diff (±SE) vs. placebo	-	19.13±7.49	4.13±7.60	14.55±7.54
95% CI (Adj. Mean Diff)	-	-0.44-38.69	-15.7-23.97	-5.12-34.23
ANCOVA (adjusted P value)		0.06	0.95	0.22



	Placebo	LL001	LH001	PPKID7
Mean difference	-2.64±2.43	-14.90±4.42	-6.09±3.04	-4.44±3.08
Adj. mean diff (±SE) vs. placebo	-	5.51±4.57	0.65±4.52	-0.72±4.47
95% CI (Adj. Mean Diff)	-	-6.43-17.44	-11.14-12.45	-12.40-10.96
ANCOVA (adjusted P value)		0.63	0.99	0.99



	Placebo	LL001	LH001	PPKID7
Mean difference	-0.05±0.04	-0.06±0.04	-0.04±0.03	-0.04±0.04
Adj. mean diff (±SE) vs. placebo	-	0.02±0.05	0.01±0.05	-0.02±0.05
95% CI (Adj. Mean Diff)	-	-0.11-0.16	-0.13-0.14	-0.16-0.12
ANCOVA (adjusted P value)		0.98	1.00	0.98



	Placebo	LL001	LH001	PPKID7
Mean difference	0.16±3.86	-4.19±5.54	-4.60±3.77	-7.28±6.72
Adj. mean diff (±SE) vs. placebo	-	-4.87±6.68	1.38±6.68	3.78±6.62
95% CI (Adj. Mean Diff)	-	-22.30-12.57	-16.05-18.81	-13.51-21.67
ANCOVA (adjusted P value)		0.89	0.99	0.94

Fig. 2 (See legend on next page.)

(See figure on previous page.)

Fig. 2 Changes in parameters related to liver function. (**A**) Alanine transaminase. (**B**) Aspartate transaminase. (**C**) Albumin. (**D**) ɣ-Glutamyltransferase. The differential values (mean difference and mean difference from placebo) are expressed as the mean ± SEM and raw data. Bars represent mean changes from baseline values and are expressed as mean ± SEM. Mann-Whitney U-test or unpaired t-test was performed to compare differences between groups. *P* values are shown in the table below. The lines represent the raw values before and 8 weeks after taking the drug. In a box and whisker plot, the whiskers represent the maximum and minimum values. Changes from baseline within groups were confirmed by performing Wilcoxon signed-ranks tests or paired t-tests. Changes over 8 weeks in the 4 groups were analyzed by Kruskal–Wallis or one-way ANOVA test. Group comparisons were made using Tukey's corrections for multiple testing. Account for baseline variability between treatment groups and calculated adjusted mean differences (± standard error) and corresponding 95% confidence intervals for each treatment group compared to the placebo group by an analysis of covariance (ANCOVA) model. Placebo group, $n = 13$; LL001 group, $n = 14$; LH001 group, $n = 15$; PPKID7 group, $n = 15$ for all parameters. All tests were two-tailed. $^*P < 0.05$

to placebo. Among the test drugs, LL001 showed a significant decrease ($P = 0.001$) in AST levels compared to baseline, and this change was significant ($P = 0.02$) compared to the placebo group (Fig. 2B). Baseline-adjusted ANCOVA analysis did not reveal any significant differences between the Probiotics group and the Placebo group in ALT and AST level, but the ALT level showed the greatest difference in LL001 (Adjust $P = 0.06$).

Albumin tended to decrease in general, but there was no significance and the average values of groups were within the normal range (3.5 ~ 5.2 g/dL; Fig. 2C). There was no significant difference in the ɣ-Glutamyltransferase (ɣGT) levels between the groups. Participants in the three groups who received test drugs were not significantly affected in the γGT levels, although a decrease (LL001, -4.19 ± 5.54 U/L; LH001, -4.60 ± 3.77 U/L; PPKID7, -7.28 ± 6.72 U/L) compared to baseline was observed (Fig. 2D). Baseline-adjusted ANCOVA analysis showed no significant differences in albumin and γGT levels between the probiotic and placebo groups.

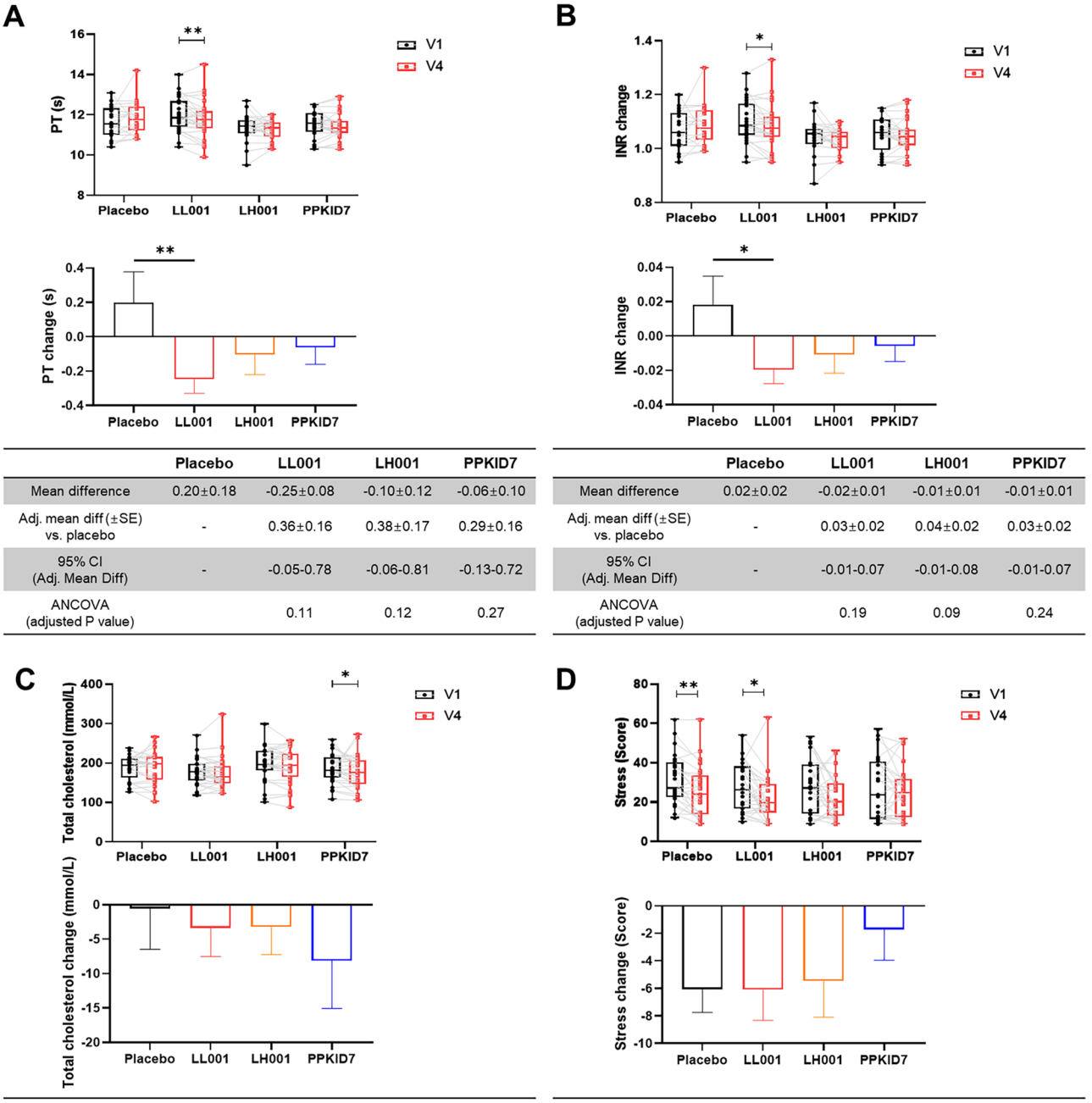
We also measured PT and identified that value was increased (0.20 ± 0.18 s) within the normal range in the placebo group (Fig. 3A). All groups treated with probiotics improved PT (LL001, -0.25 ± 0.08 s; LH001, -0.10 ± 0.12 s; PPKID7, -0.06 ± 0.10 s). In particular, the LL001 group showed significant improvement ($P = 0.007$) compared to baseline after 8 weeks, and the PT parameter improved significantly compared to the placebo group ($P = 0.007$). INR parameters were also observed to have similar effects to PT, with the LL001 group showing a significant improvement in INR compared to baseline ($P = 0.02$) within the group, and placebo ($P = 0.02$, Fig. 3B). In baseline-adjusted ANCOVA analysis, PT and INR did not differ significantly between the placebo and probiotic groups (Fig. 3A, B). All groups were observed decreased in total cholesterol level (placebo, -0.57 ± 5.92 mmol/L; LL001, -3.40 ± 4.13 mmol/L; LH001, -3.25 ± 3.99 mmol/L; PPKID7, -8.14 ± 6.93 mmol/L; Fig. 3C). The PPKID7 group showed a significant improvement ($P = 0.03$) in total cholesterol levels after 8 weeks compared to baseline. The changes in the other groups did not reach statistical significance. In addition, participants' stress was measured through a survey related to changes in quality of life. All groups showed a decrease in stress scores after the clinical trial, but only the placebo group ($P = 0.001$) and LL001 ($P = 0.01$) group reached significance after 8 weeks (Fig. 3D). In baseline-corrected ANCOVA analysis, TCHO and Stress did not differ significantly between the Placebo and Probiotic groups (Fig. 3C, D).

Secondary outcome: stool microbiome by probiotics treatment

Changes in microbial diversity and differences between microbial communities were evaluated using alpha diversity indices (Chao 1 index and Shannon index) and beta diversity index (Principal Coordinate Analysis) (Fig. 4A and B). Interestingly, the diversity and changes in the stool microbiome of the four groups showed no significant differences at baseline. Moreover, at the end of the intervention, changes in the gut microbiota composition between the four groups were not significant.

Compared to the placebo group, the test drug treatment induced changes in the proportion of the dominant microbiota (Fig. 4C and Fig. S3). At the phylum levels, dominant microbiota proportion changes were observed in all groups over 8 weeks of treatment. Especially, the participants receiving LL001 and LH001 decreased the proportion of *Proteobacteria* (LL001, -9.28 ± 6.01%, $P = 0.33$, FDR $P = 0.09$; LH001, -3.87 ± 6.03%, $P = 0.95$, FDR $P = 0.53$) compared to baseline (Supplemental Fig. S4). The difference due to treatment of the test drugs were also confirmed in the Firmicutes/Bacteroidetes (F/B) ratio (Fig. 4C). Placebo increased the change in F/B ratio (0.57 ± 0.59), but placebo had no effect. In contrast, a decrease was observed in the test drug groups (LL001, -0.62 ± 0.65; LH001, -1.99 ± 1.37) except the PPKID7 group (Fig. 4C).

Changes in the relative abundance of specific taxa in the placebo and test drug treatments were assessed (Fig. 4D). In *Ruminococcaceae* family, no change was observed in the placebo group, a slight increase was observed in the LL001 group (1.00 ± 2.01%, $P = 0.99$, FDR $P = 0.76$), but a decrease was observed in the LH001 group (-2.42 ± 3.74%, $P = 0.95$, FDR $P = 0.52$)



	Placebo	LL001	LH001	PPKID7
Mean difference	0.20±0.18	-0.25±0.08	-0.10±0.12	-0.06±0.10
Adj. mean diff (±SE) vs. placebo	-	0.36±0.16	0.38±0.17	0.29±0.16
95% CI (Adj. Mean Diff)	-	-0.05-0.78	-0.06-0.81	-0.13-0.72
ANCOVA (adjusted P value)		0.11	0.12	0.27

	Placebo	LL001	LH001	PPKID7
Mean difference	0.02±0.02	-0.02±0.01	-0.01±0.01	-0.01±0.01
Adj. mean diff (±SE) vs. placebo	-	0.03±0.02	0.04±0.02	0.03±0.02
95% CI (Adj. Mean Diff)	-	-0.01-0.07	-0.01-0.08	-0.01-0.07
ANCOVA (adjusted P value)		0.19	0.09	0.24

	Placebo	LL001	LH001	PPKID7
Mean difference	-0.57±5.92	-3.40±4.13	-3.25±3.99	-8.14±6.93
Adj. mean diff (±SE) vs. placebo	-	-4.87±6.68	1.38±6.68	3.78±6.62
95% CI (Adj. Mean Diff)	-	-22.30-12.57	-16.05-18.81	-13.51-21.07
ANCOVA (adjusted P value)		0.89	0.99	0.94

	Placebo	LL001	LH001	PPKID7
Mean difference	-6.08±1.68	-6.1±2.22	-5.47±2.63	-1.72±2.24
Adj. mean diff (±SE) vs. placebo	-	1.91±2.69	1.11±2.75	-1.88±2.74
95% CI (Adj. Mean Diff)	-	-5.10-8.92	-6.07-8.28	-9.03-5.26
ANCOVA (adjusted P value)		0.89	0.98	0.90

Fig. 3 (See legend on next page.)

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Fig. 3 Changes in parameters related to blood clot. (**a**) Prothrombin. (**b**) International normalized ratio. (**C**) cholesterol. (**D**) stress. The differential values (mean difference and mean difference from placebo) are expressed as the mean ± SEM and raw data. Bars represent mean changes from baseline values and are expressed as mean ± SEM. Mann-Whitney U-test or unpaired t-test was performed to compare differences between groups. *P* values are shown in the table below. The lines represent the raw values before and 8 weeks after taking the drug. In a box and whisker plot, the whiskers represent the maximum and minimum values. Changes from baseline within groups were confirmed by performing Wilcoxon signed-ranks tests or paired t-tests. Changes over 8 weeks in the 4 groups were analyzed by Kruskal–Wallis or one-way ANOVA test. Group comparisons were made using Tukey's corrections for multiple testing. Account for baseline variability between treatment groups and calculated adjusted mean differences (± standard error) and corresponding 95% confidence intervals for each treatment group compared to the placebo group by an analysis of covariance (ANCOVA) model. Placebo group, $n = 13$; LL001 group, $n = 14$; LH001 group, $n = 15$; PPKID7 group, $n = 15$ for all parameters. All tests were two-tailed. *$P < 0.05$

and PPKID7 group (-4.78 ± 2.24%, $P = 0.47$, FDR $P = 0.14$). In *Lachnospiraceae* family, a decrease was observed in the probiotic treatment groups after 8 weeks (LL001, -1.87 ± 2.43%; LH001, -2.00 ± 1.48%; PPKID7, -2.30 ± 2.62%). But there was no significant change (LL001, $P = 0.90$, FDR $P = 0.45$; LH001, $P = 0.92$, FDR $P = 0.47$; PPKID7, $P = 0.80$, FDR $P = 0.33$). After 8 weeks, the placebo group showed an increase in abundance of *Blautia* genus compared to the baseline (0.97 ± 0.20%, $P = 0.95$, FDR $P = 0.54$). The participants who received the test drugs were not affected. Only the PPKID7 group showed a slight decrease (-0.50 ± 0.23%, $P = 0.23$, FDR $P = 0.06$). Changes in *Blautia* abundance after 8 weeks of administration were significantly different between the LL001 ($P = 0.01$, FDR $P = 0.03$), LH001 ($P = 0.04$, FDR $P = 0.01$) and PPKID7 ($P < 0.01$, FDR $P < 0.01$) groups and the placebo group.

We confirmed the changes in abundance of *Bifidobacterium* and *Lactobacillus* genera, and compared and analyzed by converting proportions to log values due to the imbalance in abundance values caused by outliers. The placebo group had no effect on changes in the abundance of *Bifidobacterium* (0.22 ± 0.39%, log2(V4/V2): -0.53 ± 0.66, $P = 0.95$, FDR $P = 0.54$) and *Lactobacillus* (0.05 ± 0.04%, log2(V4/V2): -0.85 ± 0.49, $P = 0.85$, FDR $P = 0.38$) (Fig. 5A and B). The abundance of the *Bifidobacterium* genus increased in the LL001 (1.02 ± 1.52%, log2(V4/V2): 1.54 ± 0.61, $P = 0.75$, FDR $P = 0.30$) and LH001 (0.47 ± 0.46, log2(V4/V2): 0.52 ± 0.90, $P = 0.98$, FDR $P = 0.63$) groups compared to that at baseline. In the *Lactobacillus* genus, LL001 treatment had an effect on the increase compared to baseline (0.40 ± 0.23%, log2(V4/V2): 1.42 ± 1.85, $P = 0.96$, FDR $P = 0.56$). Differences between groups before and after treatment were analyzed using LEfSe analysis (Fig. 5C). In the placebo group, *Prevotella-massilia timonensis* species of *Alloprevotella* genus was highest before treatment, but after treatment, the unclassified species PAC001136_*s* of *Clostridium* genus was the highest. LL001 treatment significantly increased the abundance of certain taxa after 8 weeks. Unclassified subtaxa of the *Prevotellaceae* family and *Lactobacillus delbrueckii* group species were the most abundant. The LH001 group had the highest number of unclassified species PAC001042_*s* of the *Prevotella* genus before treatment. After LL001 treatment, the highest level of *Bifidobacterium* genus of the *Actinobacteria* phylum was observed. In the PPKID group, the highest level of *Eubacterium hallii*, a subspecies of the genus *Eubacterium*_g5, was observed before the treatment. After PPKID7 treatment, high levels of *Citrobacter* and *Ruminococcus torques* species were observed (Fig. 5C).

LEfSe analysis was used to determine differences between the placebo and probiotic groups after treatment (Supplementary fig S5). In a comparison between the placebo group and LL001 group, *Bacteroides* species were dominant in the placebo group, whereas *Lactobacillus delbrueckii* and *Bifidobacterium bifidum* were dominant in LL001. In a comparison between the placebo group and LH001, *Sutterella wadsworthensis* and *Parabacteroides distasonis* were dominant in the placebo group, whereas *Bacteroides uniformis* was most dominant in LH001. In the comparison between the placebo group and PPKID7, *Ruminococcus lactaris* was dominant in the placebo group, whereas *Bacteroides salyersiae* was most dominant in PPKID7.

The differences in the top 20 genera before and after administration were compared and analyzed for each group using a chord diagram (Fig. 6). Compared to the placebo group, the probiotic groups showed an increase in the genus *Bacterodies* (Placebo, V1: 37.15% V4: 36.72%; LL001, V1: 31.62% V4: 39.32%; LH001, V1: 19.17% V4: 34.35%; PPKID7, V1: 36.68% V4: 41.83%) after administration. Additionally, the genera *Escherichia* (Placebo, V1: 5.80% V4: 4.77%; LL001, V1: 3.07% V4: 3.29%; LH001, V1: 2.25% V4: 3.56%; PPKID7, V1: 1.92% V4: 2.87%), *Dialister* (Placebo, V1: 2.28% V4: 2.17%; LL001, V1: 1.03% V4: 3.28%; LH001, V1: 1.30% V4: 2.49%; PPKID7, V1: 1.78% V4: 3.69%), and *Bifidobacterium* (Placebo, V1: 0.64% V4: 0.86%; LL001, V1: 0.99% V4: 2.01%; LH001, V1: 0.38% V4: 0.85%; PPKID7, V1: 0.69% V4: 1.66%) showed an increase after 8 weeks. Conversely, the genus *Enterobacteriaceae_g* (Placebo, V1: 0.22% V4: 0.07%; LL001, V1: 5.91% V4: 1.32%; LH001, V1: 6.82% V4: 2.29%; PPKID7, V1: 0.85% V4: 0.43%) showed a decrease in the probiotic groups after 8 weeks.

The results of examining changes in KEGG pathways following administration revealed that, with the

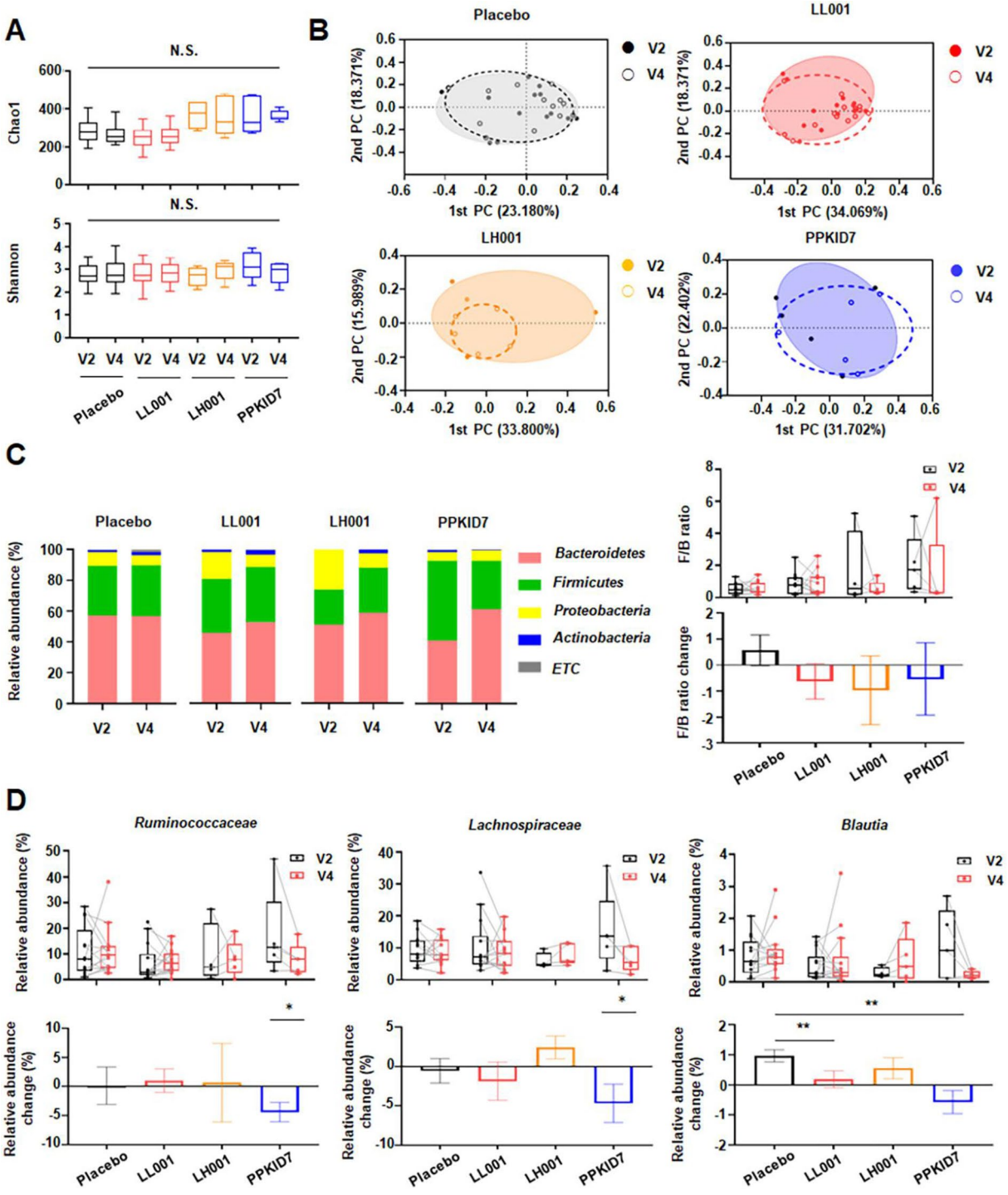

Fig. 4 Changes in stool microbiome. (**A**) Species richness index (Chao1) and species evenness index (Shannon). Placebo group, $n=13$; LL001 group, $n=14$; LH001, $n=11$; PPKID, $n=15$. (**B**) Principal Coordinates Analysis of participants' stool microbiota composition at baseline and end point of the trial. Stool microbiota dissimilarity between samples is represented by principal coordinates analysis (genus-level Generalized UniFrac distance). Placebo group, $n=13$; LL001 group, $n=14$; LH001, $n=11$; PPKID, $n=15$. (**C**) Relative abundance of bacterial phylum level and change in F/B ratio obtained by 16 S rDNA sequencing of participants' stool samples. (**D**) Change in specific taxa relative abundance. The differential values (mean difference and mean difference from placebo) are expressed as the mean ± SEM and raw data. Bars represent mean changes from baseline values and are expressed as mean ± SEM. Mann-Whitney U-test or unpaired t-test was performed to compare differences between groups. The lines represent the raw values before and 8 weeks after taking the drug. In a box and whisker plot, the whiskers represent the maximum and minimum values. Changes from baseline within groups were confirmed by performing Wilcoxon signed-ranks tests or paired t-tests. Changes over 8 weeks in the 4 groups were analyzed by Kruskal–Wallis or one-way ANOVA test. Group comparisons were made using Tukey's corrections for multiple testing. Benjamini and Hochberg false discovery rate (FDR) was performed to correction for multiple testing. Placebo group, $n=13$; LL001 group, $n=14$; LH001, $n=11$; PPKID, $n=15$

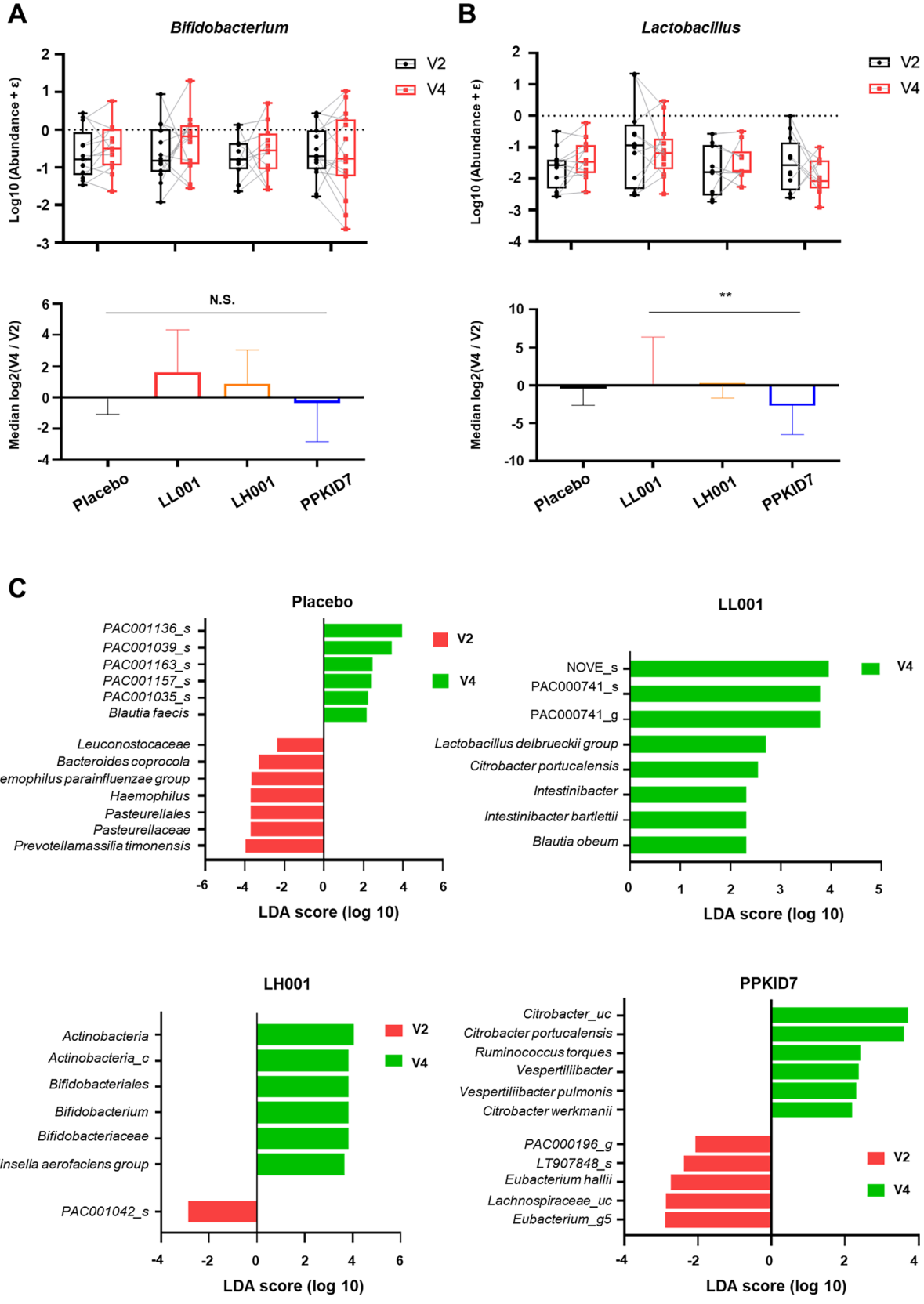

Fig. 5 (See legend on next page.)

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Fig. 5 Change in *Bifidobacterium* genus and *Lactobacillus* genus, and LEfSe analysis. (**A**) *Bifidobacterium* genus. (**B**) *Lactobacillus* genus. (**C**) LEfSe analysis. Taxonomic abundance was calculated by log10 transformation. Changes from baseline were calculated by log2 transformation (log2V4/log2V2). The differential values are expressed as the multiple and raw data. Bars represent log-transformed changes from baseline values and are expressed as meandian with 95% CI. Mann-Whitney U-test or unpaired t-test was performed to compare differences between groups. The lines represent the raw values before and 8 weeks after taking the drug. In a box and whisker plot, the whiskers represent the maximum and minimum values. Changes from baseline within groups were confirmed by performing Wilcoxon signed-ranks tests or paired t-tests. Changes over 8 weeks in the 4 groups were analyzed by Kruskal–Wallis or one-way ANOVA test. Group comparisons were made using Tukey's corrections for multiple testing. Benjamini and Hochberg false discovery rate (FDR) was performed to correction for multiple testing. Placebo group, $n=13$; LL001 group, $n=14$; LH001, $n=11$; PPKID, $n=15$. (**c**) LEfSe analysis histograms of differential species in groups. Taxa enriched in V2 are indicated with by negative LDA scores (red), and taxa enriched in V4 are indicated with positive LDA scores (green). Only taxa with an LDA score > 2 at $P<0.05$ are shown

exception of LH001, no significant pathways were observed to increase after 8 weeks in any of the other groups (Supplementary Fig. S6). LH001 administration resulted in increases in genes related to lysosomes (ko04142), glycosaminoglycan degradation (ko00531), glycosphingolipid biosynthesis (ko00604), other glycan degradation (ko00511), and steroid hormone biosynthesis (ko00140).

Safety

Of the total sample of 103 patients, two, three, four, and six patients in the placebo, LL001, LH001, and PPKID7 groups, respectively, showed adverse events. No severe adverse events were observed. No difference in the incidence of AEs (total and serious AE) was observed among the four groups (Table 2).

Discussion

This randomized controlled trial assessed the impact of 8 weeks of supplementation with probiotics LL001, LH001, and PP7KID on the stool microbiome outcome in adults with MASLD. Previous reports have demonstrated the effects of probiotics in patients with MASLD. However, the main evaluation endpoints were mostly liver function tests in blood. Few clinical trials have evaluated and analyzed the changes in the gut microbiome after probiotic treatment.

Probiotics (LL001, *Lactobacillus lactis*; LH001, *Lactobacillus helveticus*; PPKID7, *Pediococcus pentosaceus*) treated for test drugs were tested for their effectiveness in preclinical studies using animal models. There was no direct correlation between the three probiotics and clinical parameters such as body weight and BMI. However, improvements in blood parameters were confirmed. Treatment with LL001 resulted in observed reductions in ALT, AST, and albumin levels, and especially improvements in PT and INR. A significant difference in ALT levels compared to the placebo group confirmed that LL001 was effective in improving liver function parameters. Improvements in AST, total cholesterol, and γGT levels were also confirmed after treatment with PPKID7. Previously reported clinical studies have confirmed that probiotic administration can have beneficial effects on serum ALT, AST, and GGT levels [31, 32]. Clinical parameters demonstrated a beneficial association between treatment with our clinically selected probiotics and liver function.

Several studies have identified changes in gut microbiota composition, intestinal barrier dysfunction, and changes in gut-derived metabolites in the gut of MASLD patients [33, 34]. Although the results on changes in gut microbiota composition were inconsistent in each study due to differences in conditions and methods, the common key factors are that gut dysbiosis worsens the progression of MASLD and that the pathological progression of MASLD continuously contributes to changes in gut microbiota composition [35].

We identified changes in the gut microbiota composition by analyzing participant stool samples and evaluated the effects of probiotic treatment. In our study, shifts and changes in the diversity of the gut microbiota community were not observed after probiotic treatment, but changes in the compositional ratio and abundance of specific taxa were confirmed. In particular, *Proteobacteria* decreased after probiotic treatment, and a decrease in the ratio of *Firmicutes* to *Bacteroidetes* was observed compared with that in the placebo group. Previous observational studies have shown an increase in *Proteobacteria* during MASLD, which is positively correlated with the progression of MASLD [36–38]. Chen et al. found that the proportion of *Bacteroidetes* was significantly reduced, whereas *Proteobacteria* was highly abundance in MASLD patients [39], and our results suggested that probiotics treatment improved the proportional imbalance of gut microbiota. Changes in taxa observed in previously reported clinical studies confirmed a phylogenetic-level decrease in *Ruminococcaceae* and *Lachnospiracea* [40, 41]. In our results, only LH001 and PPKID7 showed a decreasing trend at the *Ruminococcaceae* level, while all groups showed a decreasing trend at the *Lachnospiraceae* level. It was confirmed that the small number of participants in the group may have an impact. In addition, as the subjects of previously reported clinical studies showed differences in the severity of MASLD, it is assumed that significant changes were not observed in the subgroup taxa as

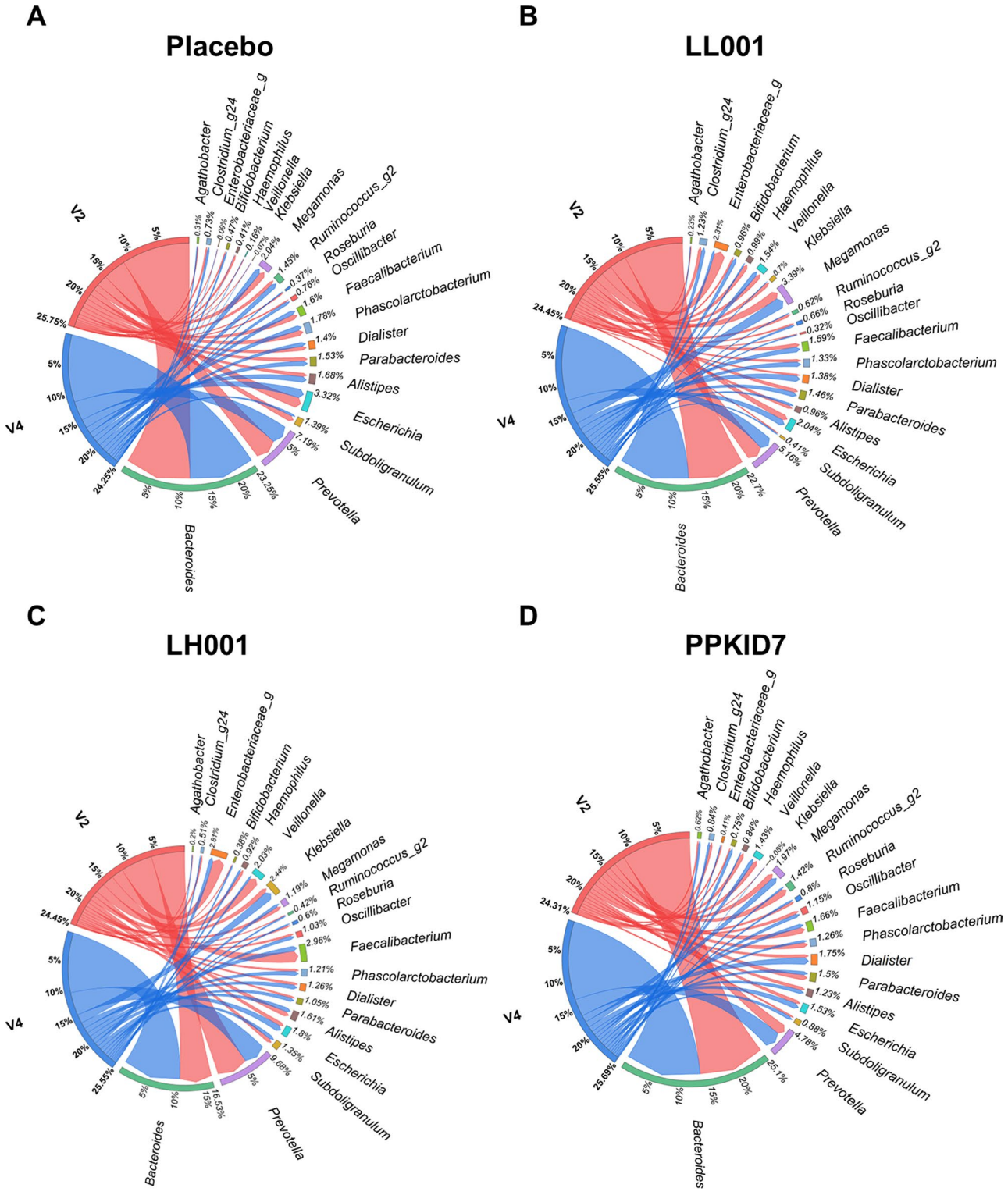

Fig. 6 Relative relation between probiotics treatment and microbiotas using a circular phylogenetic tree. (**A**) Placebo. (**B**) LL001. (**C**) LH001. (**D**) PPKID7

the disease in our clinical participants was in the early stage. Additional large-scale clinical studies are needed for clear data analysis and clarification.

Blautia was reported to be more abundant in patients compared to controls [37]. In our results, it was observed that probiotic treatment inhibited the increase

Table 2 Adverse events

Variable (*n* [%])	Placebo group		LL001 group		LH001 group		PPKID7 group		
	AE	Serious AE	AE	Serious AE	AE	Serious AE	AE	Serious AE	*P*-value [a]
Abdominal pain	1 (4)						1 (3)		NS
Bloating			1 (3)						NS
Constipation			1 (3)		3 (12)		2 (7)		NS
Diarrhea	1 (4)				1 (4)		1 (3)		NS
Nausea			1 (3)						NS
Urticaria							1 (3)		NS
Total	2 (8)		3 (9)		4 (16)		6 (16)		NS

n, number; AE, adverse event; NS, not significant

[a] P value for the difference among placebo, LL001, LH001, and PPKID7 groups

in *Blautia*. In an animal study that induced MASLD through a Western-style diet, it was reported that *Blautia producta* contributes to MASLD [42]. Our results suggest that probiotic treatment may be helpful in preventing the progression of non-alcoholic fatty liver disease caused by *Blautia*. In our results, it was observed an increase in the abundance of the dominant genus, *Bacteroides*, in the probiotic group. Several studies have shown that Bacteroides has a strong potential to prevent the onset and progression of MASLD [43, 44]. Furthermore, a decrease in *Bacteroides* abundance was observed in MASLD patients [17, 45]. *Bacteroides* has been reported to enhance immune function, reduce liver inflammation, and improve metabolic dysfunction [46, 47]. Our findings suggest that probiotic treatment may help prevent MASLD through its effects on *Bacteroides*.

There is growing evidence that the gut-liver axis plays an important role in the pathophysiology of liver disease, and probiotics may affect the improvement of liver disease through modulation of the gut microbiota [48, 49]. Modulation of the gut microbiota can also have linked by changes in gut microbiota-derived metabolites, including tryptophan metabolites and short chain fatty acids (SCFAs) [50–52].

No serious AEs were observed in this study, and the AEs did not stop the study. Furthermore, considering that there were no differences in the incidence of AEs among the four groups, it can be concluded that next-generation probiotic treatment is safe and well tolerated in patients with MASLD.

Our study was designed as a clinical trial to confirm the safety and stability of probiotics, the effects of which have been proven in animal models, while also testing their effects on liver function parameters through gut microbiota analysis. Since probiotics are widely available and accessible supplements, we expected that if probiotics were used to treat MASLD, they would have a positive effect on disease improvement in the early stages of the disease.

Additionally, we predicted that probiotics would change their impact on the gut microbiome. We did not control for intentional lifestyle interventions and only confirmed the effects of probiotics alone. The scale of clinical trials for accurate evaluation, correlation with clinical parameters of changes in the gut microbiota on host metabolism are additional limitations that need to be addressed.

This study has limitations that should be acknowledged. First, the duration of probiotic administration and follow-up was relatively short, making it difficult to determine the long-term efficacy and sustainability of the observed effects. Second, this study did not include a placebo-only arm due to ethical and regulatory constraints during the IRB review process. All participants received Legalon® (silymarin) as background therapy during the study. The hepatoprotective properties of Legalon® might induce a confounding effect when evaluating the efficacy of the probiotics. However, to minimize this confounding bias, Legalon® was administered uniformly across all groups, thereby reducing variability associated with baseline liver protection. Despite these limitations, the study provides valuable preliminary evidence supporting the potential benefit of probiotic supplementation on gut–liver axis modulation in patients receiving standard hepatoprotective therapy.

Conclusion

Our randomized controlled trial suggests that treatment with probiotics may change the gut microbiome and positively impact the clinical parameters of MASLD. The changes in stool microbiota observed in our study support the need to evaluate the effects of probiotic treatment on host metabolism-related parameters in MASLD.

Abbreviations

MASLD	Metabolic dysfunction-associated steatotic liver disease
LL001	Lactobacillus delbrueckii subsp. Lactis
LH001	L. helveticus
PPKID7	Pediococcus pentosaceus KID7
AST	Aspartate transferase
ALT	Alanine transferase
γGT	Glutamyltransferase

INR	International normalized ratio
PT	Prothrombin time

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-07478-z.

Supplementary Material 1

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Author contributions

Ki Tae Suk: Conceptualization, investigation, methodology, validation, formal analysis, project administration, data curation, writing, original draft, writing, review, and editing. Sung-Min Won, Hyunchae Joung, In Gyu Park: Conceptualization, investigation, methodology, validation, project administration, writing – review and editing, data curation, and resources. and Sang Hak Han: Data curation; writing – review and editing. Young Lim Ham, Ji Sook Han, Yoojin Kwon, and Dong Joon Kim: Visualization, investigation, formal analysis, project administration, software, data curation, and resources.

Data availability

Data are available upon reasonable request. Data generated or analyzed during the study are available from the corresponding author by request.

Declarations

Patient consent for publication and ethics approval

This project followed the ethics of the 1975 Helsinki Declaration, as reflected by a prior approval by the institutional review board for human research in hospitals (2016 – 134). Informed consent was obtained from all the participants. All authors had access to the study data and reviewed and approved the final manuscript.

Conflict of interest

All authors declare no conflicts of interest.

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