Supplementary Materials for

A novel multifunctional *Lactiplantibacillus plantarum* HMPM2111: antibacterial and anti-inflammatory effects against pathogenic bacteria stresses

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Supplementary methods

1. Determination of growth curve of *L. plantarum*

The isolates stored at -80 °C were revived, activated, and incubated at 37 °C until turbidity was observed. Based on a 10% inoculum concentration, the isolates were introduced into fresh MRS liquid medium. Samples were collected at the following time points: 0, 2, 4, 6, 8, 10, 11, 12, 12.5, 13, 14, 16, 18, 20, 22, 24, 30, 36, and 48 hours. At each time point, 200 μL of the culture was transferred into a 96-well plate, and the optical density at 600 nm (OD600) was measured using a microplate reader. Each time point was assessed in triplicate.

2. Determination of acid-producing capacity of *L. plantarum*

The twelve isolated strains were inoculated into fresh MRS liquid medium at a 10% inoculum concentration and incubated at 37°C with agitation at 220 rpm for a duration of 12 hours. The pH of the cultures was assessed using a pH meter at two-hour intervals, with 10 mL aliquots collected at each time point. Each sample was subjected to triplicate measurements to ensure accuracy and reproducibility.

3. Temperature tolerance of *L. plantarum*

The isolated bacterial strains were initially cultured in MRS liquid medium with a 10% inoculum and incubated overnight at 37°C under shaking conditions. Subsequently, 1 mL of the activated bacterial culture was transferred into 9 mL of fresh MRS liquid medium. This mixture was then incubated at four different temperatures (27°C, 37°C, 55°C, and 75°C) using constant temperature incubators. Optical density at 600 nm (OD_{600}) was measured at time intervals of 0, 2, 4, 6, 8, 10, 12, and 24 hours to monitor bacterial growth.

4. Study on the antibacterial properties of *L. plantarum*

S. aureus, *P. aeruginosa*, *S. typhimurium*, *E. cloacae*, *E. coli*, *M. luteus* and 12 isolated strains of *L. plantarum* were activated. 12 strains of *L. plantarum* were cultured to the exponential stage, and the OD_{600} value was measured and the plate viable bacteria count were carried out to ensure that the inoculated strains (OD_{600}=1.8; 4×10^{11} CFU/mL). After 12 hours of culture, 6 strains of pathogenic bacteria were diluted to 5×10^{6} CFU/mL. The control group of *P. aeruginosa* and *S. typhimurium* was ofloxacin at a concentration of 400 μg/mL, and the control group of the other four pathogenic bacteria was ampicillin at a concentration of 100 μg/mL. The blank group was all uninoculated with fresh MRS medium. The inhibition rate was determined by the filter paper method, the diameter of the circular filter paper was 10mm, each filter paper was inoculated with 25 μL of the analyte, incubated at 37°C for 24 hours, and the diameter of the inhibition zone was observed and measured. The formula for calculating the inhibition rate is as following:

$$\frac{\text{the diameter of the inhibition zone in the L. plantarum group} - \text{the diameter of the filter paper}}{\text{the diameter of the inhibition zone in the positive control group} - \text{the diameter of the filter paper}} \times 100\%.$$

5. Anti-inflammatory properties of *L. plantarum*

Human normal colon epithelial cells (NCM-460) were cultured under standard conditions at 37°C with 5% CO_2. The cells were digested and counted, achieving a concentration of 3.5×10^{5} cells/mL, and subsequently plated at 2 mL per well in a 6-well plate. Three experimental groups were established: an untreated control group, a DSS model group, and an *L. plantarum* intervention group, each with three replicates. Once the cells adhered and reached approximately 60% confluency, inflammation modeling was initiated using DSS at a concentration of 6 mg/mL, with an intervention period of 12 hours. Following this, cells from each group were digested and counted. The appropriate solution was prepared by adding inactivated *L. plantarum* at a ratio of 100:1 relative to NCM-460 cells, and the cell culture medium was

adjusted to a total volume of 2 mL. After 24 hours of co-culture, the cell pellet was harvested, nucleic acids were extracted, and the expression of *IL-1β* mRNA was quantified using RT-qPCR. Additionally, the expression level of IL-1β protein was assessed using an ELISA kit.

6. Construction of oxidative stress model

Several NGM solid plate media were prepared with distinct compositions: the control group received OP50 and double-distilled water; the model group was supplemented with OP50, double-distilled water, and 0.5 mM juglone; the treatment group included OP50, double-distilled water, 0.5 mM juglone, and supernatant fermentation broth; and the *L. plantarum* HMPM2111 group contained OP50, double-distilled water, 0.5 mM juglone, and *L. plantarum* HMPM2111 solution. Subsequently, a predetermined number of C. elegans were inoculated onto each plate, followed by incubation in a biochemical incubator at 20°C for a duration of three days.

7. Complete genome sequencing of strain HMPM2111

The genome sequencing of the strain was conducted by Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China) utilizing the Illumina HiSeq 2000 (2 × 100 bp) and PacBio Sequel II platforms. For the Illumina sequencing, DNA samples were fragmented using a Covaris system, and these fragments were employed to construct Illumina sequencing libraries, which were subsequently sequenced on the Illumina HiSeq 2000. In the PacBio sequencing process, DNA samples were sheared into 8–10 kb fragments using G-tubes, which were then used to construct SMRTbell sequencing libraries. These prepared libraries were combined with the polymerase at the base of zero-mode waveguides (ZMW) and sequenced using the PacBio platform.

8. Genomic annotation

GeneMarkS identified CDS genes, IslandPathDIMOB detected genomic islands, Barrnap found rRNA genes, tRNAscan-SE located tRNA genes, and Infernal searched for sRNA genes. Minced identified CRISPR/Cas systems. Virulence genes were predicted via the VFDB database, and antibiotic resistance genes were screened using the CARD database with BLAST. The CAZy Database analyzed carbohydrate-active enzymes in two strain genomes. The genomes were annotated using the NR, COG, GO, Pfam, and Swiss-prot databases, and the metabolic map was derived from KEGG.

9. Determination of inflammatory indexes in mice

During the 17 days of culturing mice, the mice were weighed and recorded daily. The formula for calculating the rate of weight change is as following:

$$\text{Body weight gain} = \frac{daily\ body\ weight - original\ weight}{original\ weight} \times 100\%$$

After the animal experiment, the mice were fasted for 5-6 hours and euthanized by intraperitoneal injection of 350 μL of 1.25% tribromoethanol and cervical dislocation method. Record the weight of mice before execution, collect samples of colon, heart, liver, lungs, spleen, kidney, eyeball blood, feces, and measure their length, net weight, and other data. The colon and liver samples were washed with physiological saline, partially placed in tissue fixative, and partially stored in a -80 ℃ freezer.

10. Pathological observation of mouse colon tissue

Hematoxylin eosin (H&E) staining: Mouse colon tissue is fixed, embedded in wax, and sliced. The slices are dewaxed, dried at 60°C for 15 minutes, and treated with 100% xylene twice for 10 minutes each. They are rehydrated through a series of ethanol solutions (100% to 50%) and soaked in PBS and double-distilled water. Hematoxylin is applied for 5 minutes, followed by rinsing and drying. Rapid acidification is done for 1 minute, rinsed, and dried. Eosin is applied for 1 minute, rinsed, and the slices are rehydrated

through ethanol solutions (50% to 100%) and treated with 100% xylene twice for 10 minutes each. After drying the fume hood, seal it with neutral gum, inspect it under a microscope, and take photos for recording. Observe and analyze the morphology and inflammatory infiltration of colon tissues in each group.

Alcian Blue-Periodic Acid Schiff staining (AB-PAS staining): Use the Alcian Blue staining kit for slice staining and observation. Refer to the PAS kit user manual for the operating steps. Using Image J software to analyze the number of goblet cell.

11. Preparation of cell-free supernatant (CFS) of *L. plantarum* HMPM2111

An overnight culture of *L. plantarum* HMPM2111 was inoculated at a 2% concentration into MRS medium and incubated at 37°C for 48 hours. The resulting bacterial suspension was subjected to centrifugation at 8,000 g for 10 minutes. Subsequently, the cell-free supernatant (CFS) was sterilized using a 0.22 μm pore size filter and stored in appropriate containers.

12. Minimum inhibitory concentration (MIC) of CFS

Serial dilutions of CFS (10%, 20%, 30%, and 50%) were prepared in LB medium with *S. aureus* or *E. coli* at about 10^5 CFU/mL. After 24 hours of incubation at 37℃, OD_{600} was measured three times and compared to a control without CFS to assess biomass.

13. Biopreservation of apple juice

Apple juice was inoculated with *S. aureus* or *E. coli* and the concentration of the indicator strains in the tubes was 5 log CFU/mL. Sterile CFS of *L. plantarum* HMPM2111 was added to the apple juice as an intervention according to the MIC value (v/v), and it was placed in the incubation at 4℃ for 72 h. Two replicates were set up in each group, and compared with the Control group which was inoculated with the causative organisms but no CFS was added. The Blank group containing only apple juice and the CFS group with CFS added but not inoculated with pathogenic bacteria were also set up. Colony forming units

(CFU/mL) of the indicator strains were determined using the dilution smear plate method, and 3 replicates were set up for each time point.

Supplementary Figures

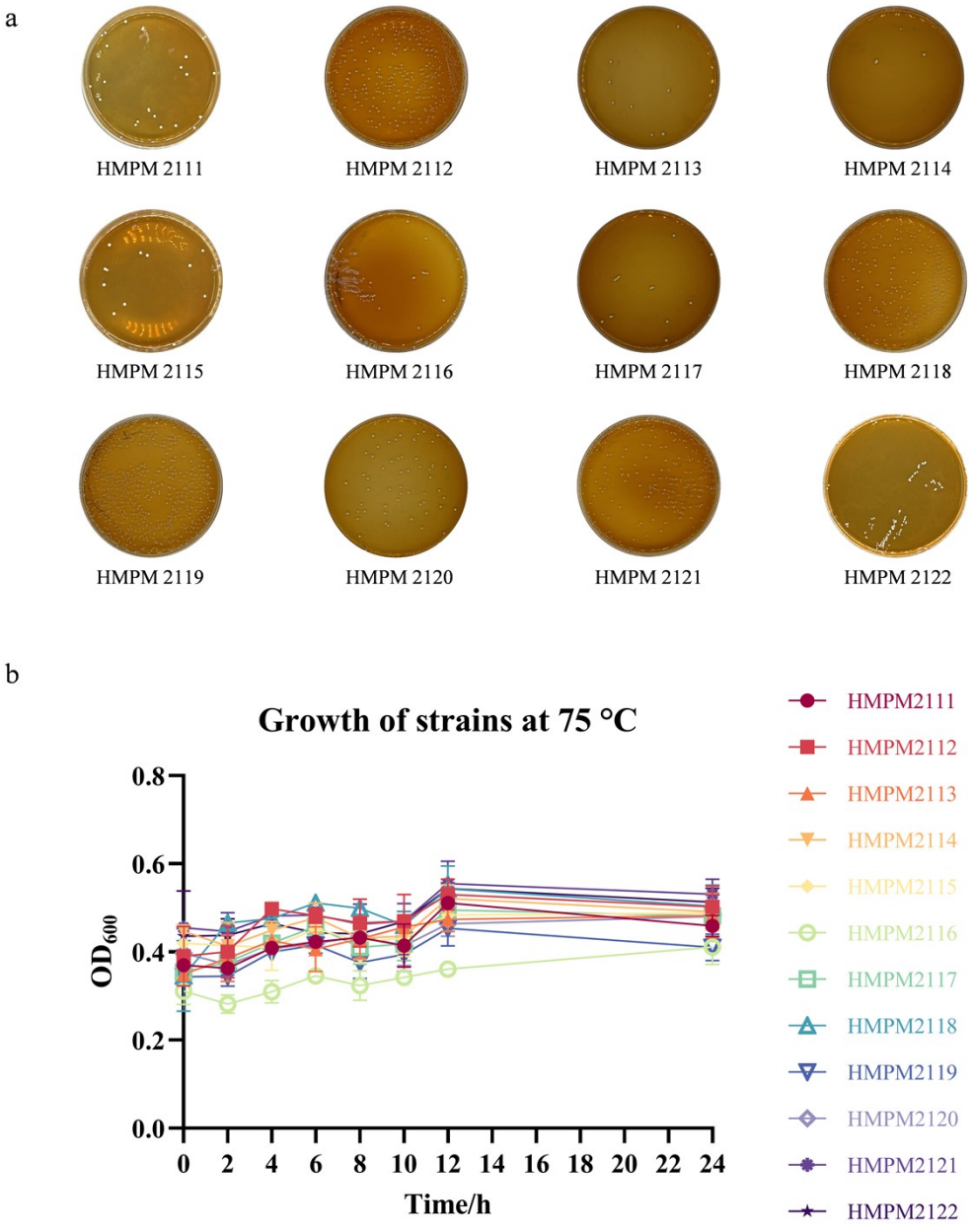

Figure S1. (a) Pictures of *L. plantarum* diluted and cultured on plate culture medium. (b) Temperature tolerance at 75 ℃ of 12 strains.

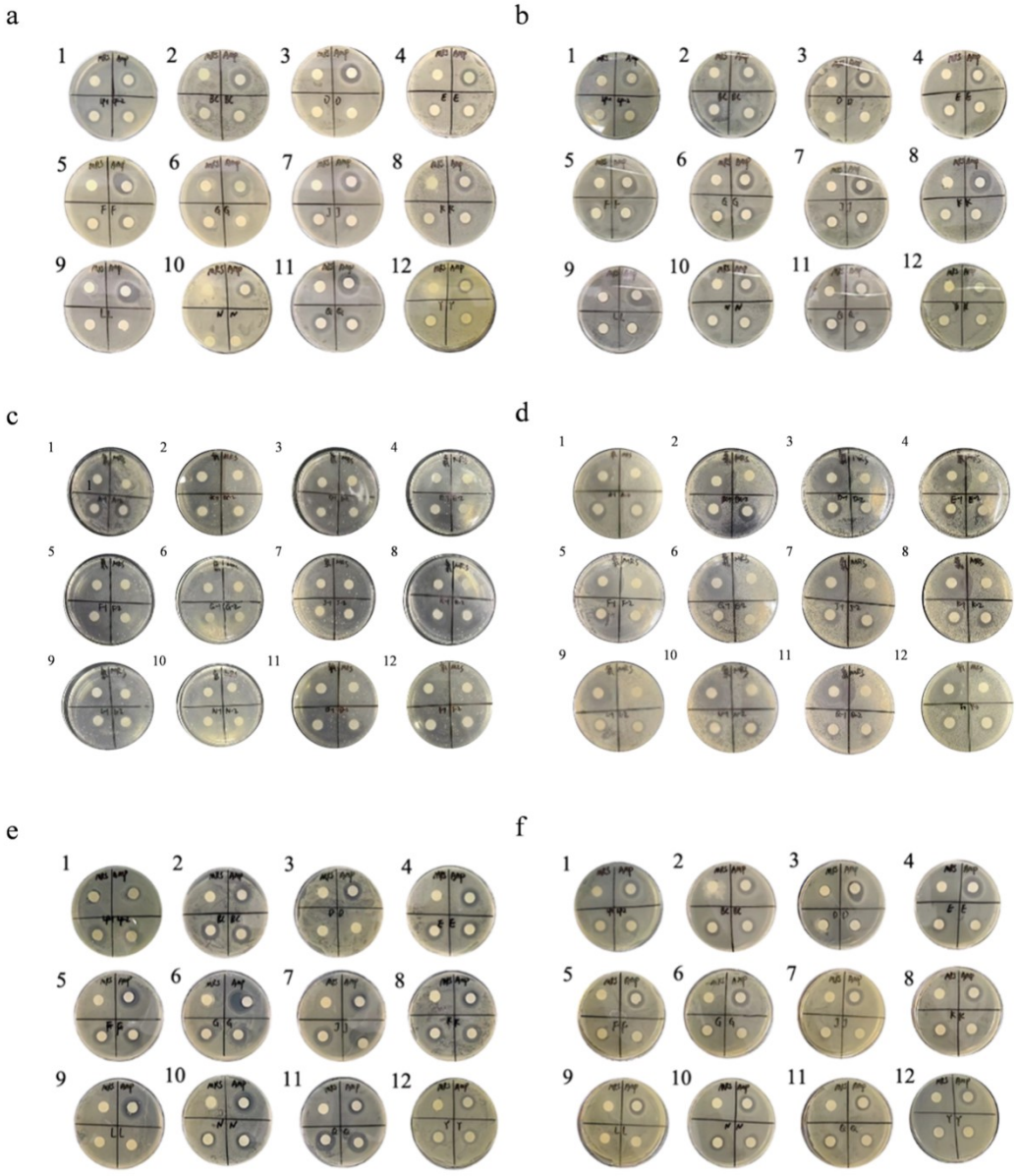

Figure S2. (a-f) Antibiotic disk diffusion test results of 12 strains *L. plantarum* respectively inhibiting *E. cloacae* (a), *M. luteus* (b), *P. aeruginosa* (c), *S. typhimurium* (d), *E. coli* (e), *S. aureus* (f). In each image, 1-12 separately represent HMPM 2111, HMPM 2112, HMPM 2113, HMPM 2114, HMPM 2115, HMPM 2116, HMPM 2117, HMPM 2118, HMPM 2119, HMPM 2120,HMPM2121,HMPM2122.

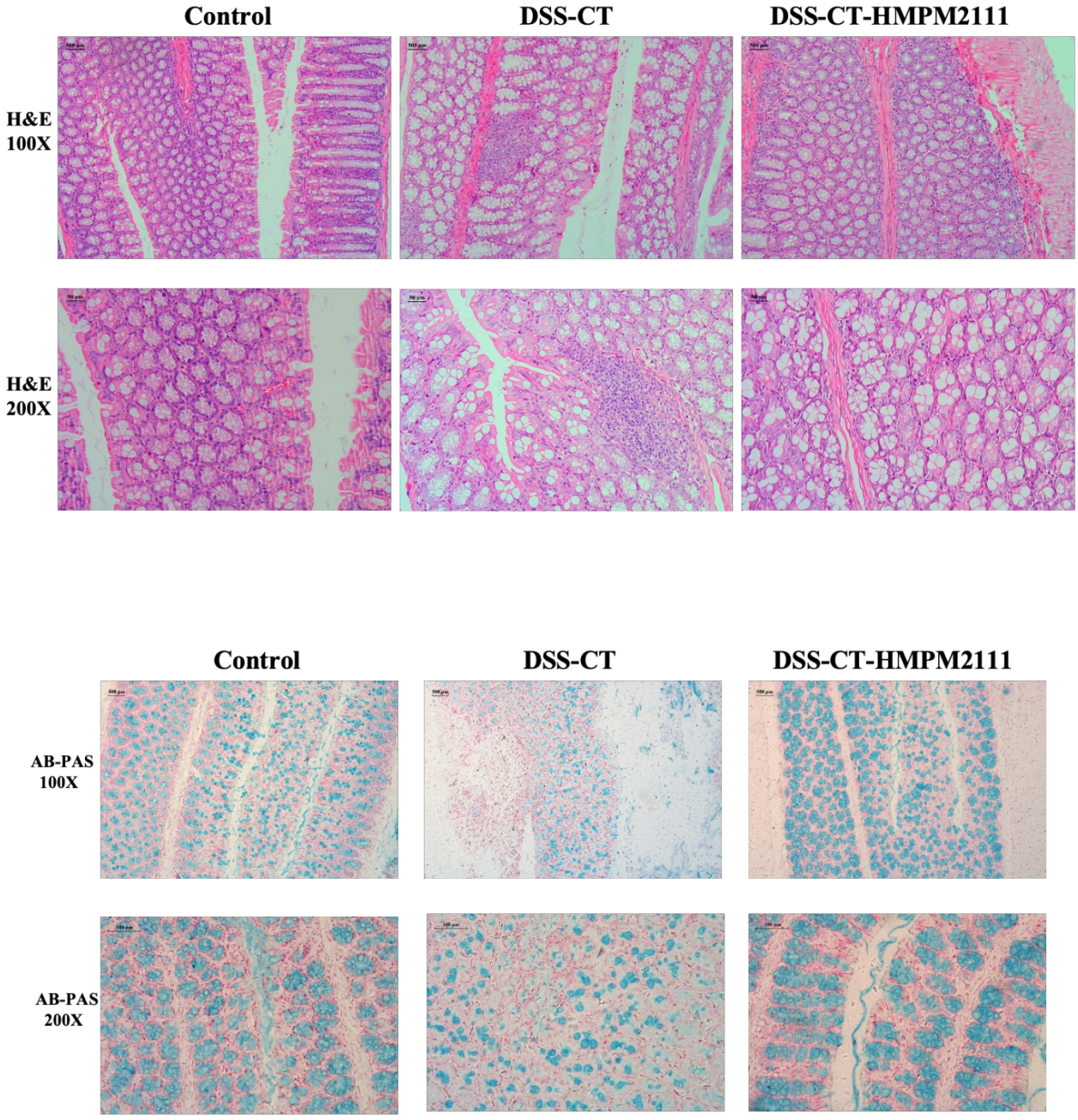

Figure S3. H&E staining and AB-PAS staining of colon tissue.

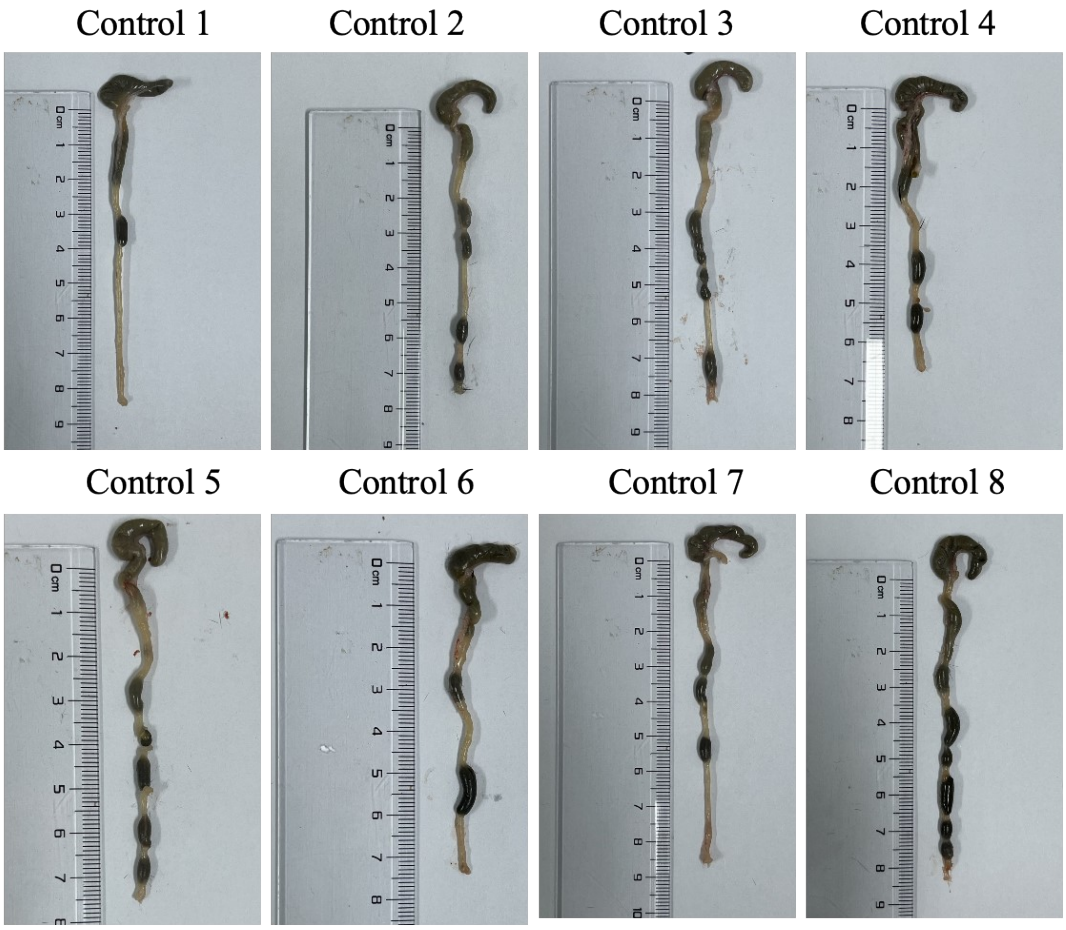

Figure S4. Picture of colons in control group.

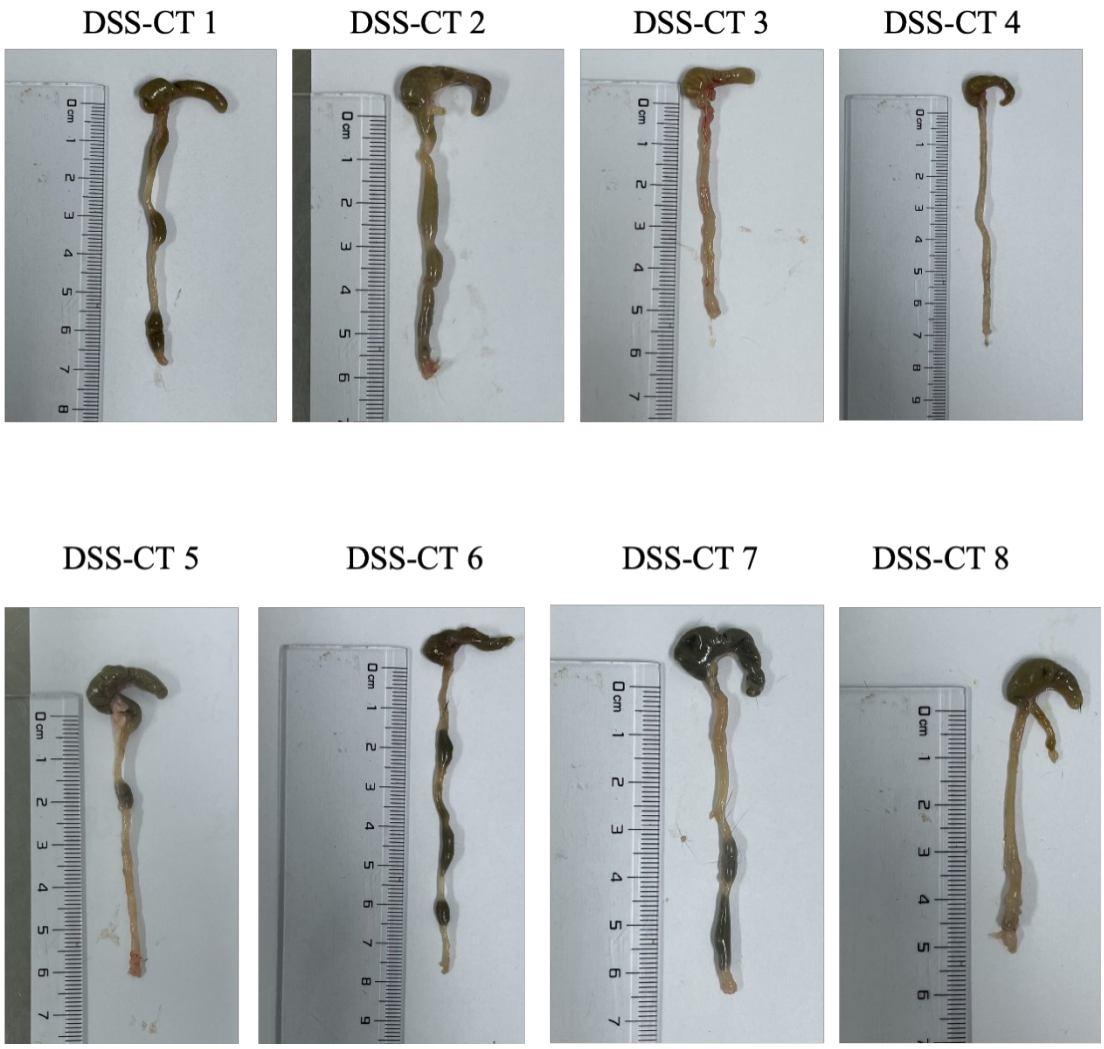

Figure S5. Picture of colons in DSS-CT group.

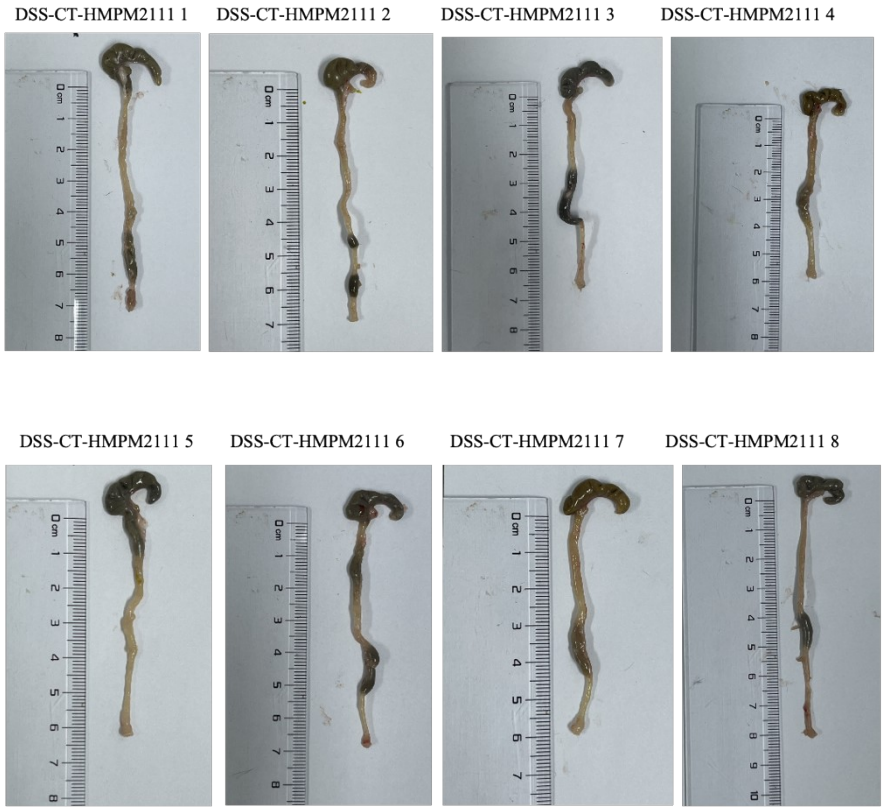

Figure S6. Picture of colons in DSS-CT-HMPM2111 group.

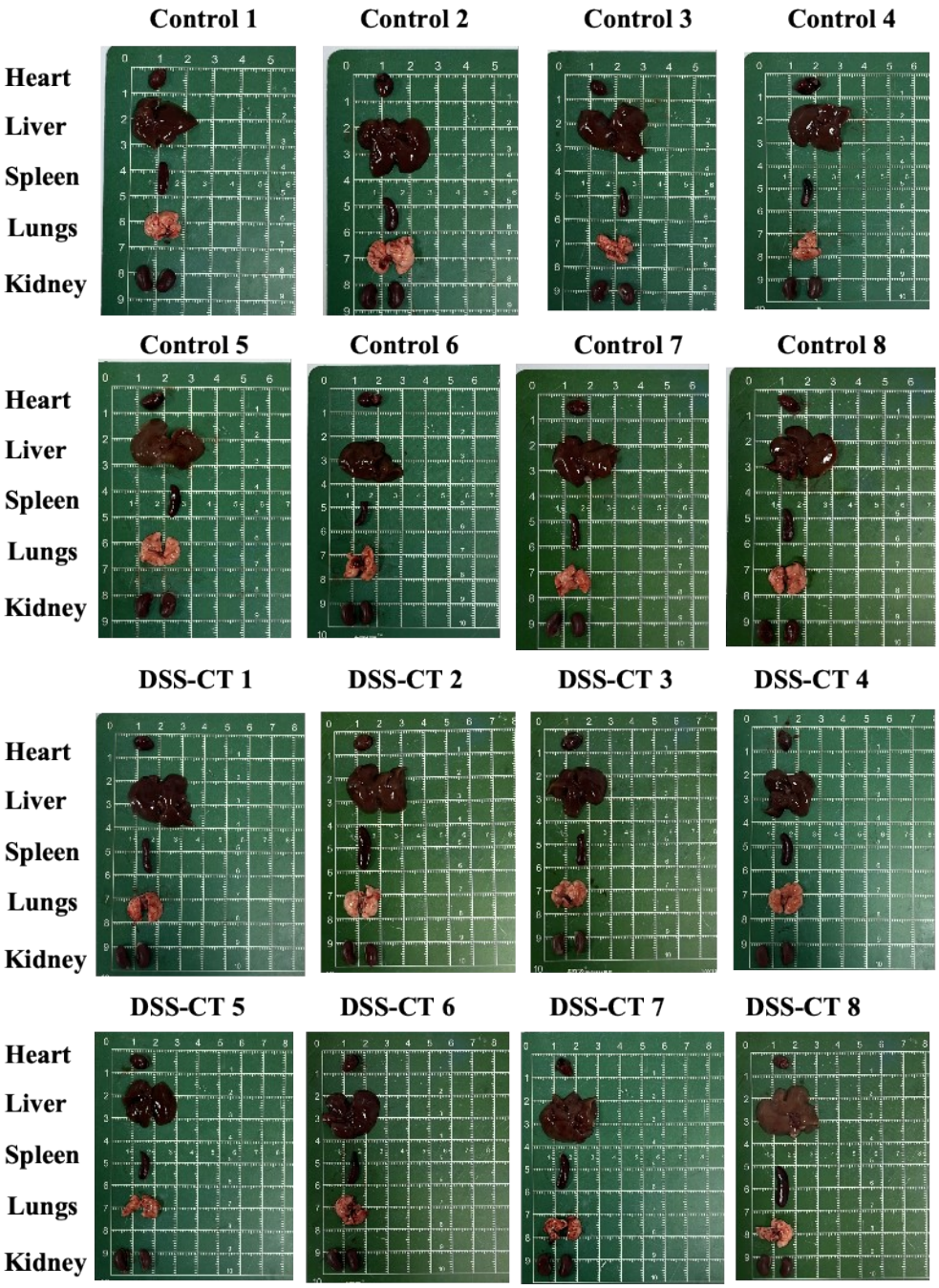

Figure S7. Picture of heart, liver, spleen, lungs and kidney in Control and DSS-CT group.

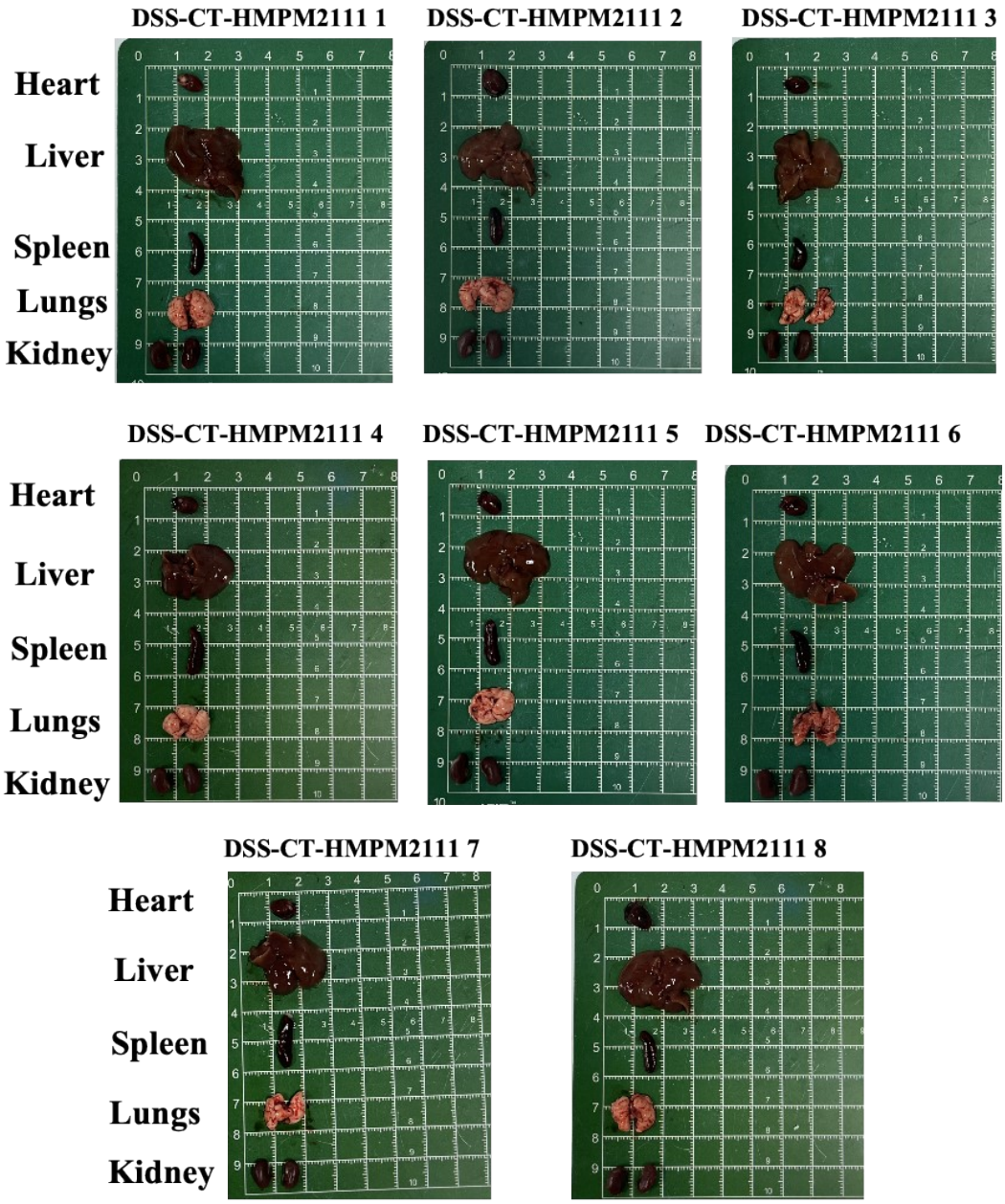

Figure S8. Picture of heart, liver, spleen, lungs and kidney in DSS-CT-HMPM2111 group.

Supplementary Table S1 Oligonucleotide primers used for RT-qPCR.

genes	description	5'-3' Primer sequence
β-actin	*β-actin*	F: CCACGTCATCAAGGAGTCAT R: GGAAGCGTAGAGGGAGAGGA
hsp-16.2	*Heat Shock Proteins-16.2*	F: CTGCAGAATCTCTCCATCTGAGTC R: AGATTCGAAGCAACTGCACC
hsp-12.6	*Heat Shock Proteins-12.6*	F: TGGAGTTGTCAATGTCCTCG R: GACTTCAATCTCTTTTGGGAGG
ctl-1	*Catalase-1*	F: CGATACCGTACTCGTGATGAT R: CCAAACAGCCACCCAAATCA
sod-3	*Superoxide Dismutase-3*	F: TGGCTAAGGATGGTGGAGAA R: GCCTTGAACCGCAATAGTGAT
ace-1	*Acetylcholinesterase-1*	F: AGTGGGCTCCTGTTCGAGAA R:CCAATAGAAAATCACCATCGACAA
hsf-1	*Heat Shock Transcription Factor-1*	F: ATGCAGCCAGGATTGTCGAA R: GCACGTTTTGAGTTGGGTCC
sod−5	*Superoxide Dismutase-5*	F: TGCCAATGCCGTTCTTCCACAG R: AACAGTTCCGAAGACAGCAGTTCC
gst−10	*Glutathione S-transferase-10*	F: CCGTCTTCTGTTCCTCGACAATGG R: CAATCTCTTGCCCGTCCACCTTC
skn−1	*skinhead-1*	F: GGTCTCCGTTGGCGTGATGATC R: CTGGTGGATGCTCGGTGAGTATTG
IL-1β	*Interleukin-1beta*	F: TGCCACCTTTTGACAGTGATG R: AAGGTCCACGGGAAAGACAC

daf-16	*decay-accelerating factor-16*	F: CAGTTCCGTGGCCAGTTCCA R: GGTCTGCTGCTGTTGAGAGG

Supplementary Table S2. Sequencing results of 12 strains *L. plantarum.*

Strain	Sequencing results
HMPM2111	TTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGACTTCATGTAGG CGAGTTGCAGCCTACAATCCGAACTGAGAATGGCTTTAAGAGATTAGCTTA CTCTCGCGAGTTCGCAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCC CAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTT GTCACCGGCAGTCTCACCAGAGTGCCCAACTTAATGCTGGCAACTGATAAT AAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTG ACGACAACCATGCACCACCTGTATCCATGTCCCCGAAGGGAACGTCTAATC TCTTAGATTTGCATAGTATGTCAAGACCTGGTAAGGTTCTTCGCGTAGCTTC GAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGA GTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTG CAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCATTCATCGTTTACGGT ATGGACTACCAGGGTATCTAATCCTGTTTGCTACCCATACTTTCGAGCCTCA GCGTCAGTTACAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATA TCTACGCATTTCACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCA AGTTTCCCAGTTTCCGATGCACTTCTTCGGTTGAGCCGAAGGCTTTCACATC AGACTTAAAAAACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAAC GCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTT TCTGGTTAAATACCGTCAATACCTGAACAGTTACTCTCAGATATGTTCTTCTT TAACAACAGAGTTTTACGAGCCGAAACCCTTCTTCACTCACGCGGCGTTGCT CCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGG AGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATTACCCTCTCAGGTCGGC TACGTATCATTGCCATGGTGAGCCGTTACCCCACCATCTAGCTAATACGCCG CGGGACCATCCAAAAGTGATAGCCGAAGCCATCTTTCAAACTCGGACCATG CGGTCCAAGTTGTTATGCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCCG CTTCTGGGCAGGTTTCCCACGTGTTACTCACCAGTTCGCCACTCACTCAAAT GTAAATCATGATGCAAGCACCAATCAATAC
HMPM2112	AAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGC ATAACAACTTGGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGCTATCA CTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACC ATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACT GAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAA TGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGC TCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGT ATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGC GGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAG CGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAA GTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCC ATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGC GGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAAC AGGATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTT GGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTG

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TGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTG
TAACACCCAAAGTCGGTGGGGTAACCTT

Supplementary Table S3 Genome features of *L. plantarum* HMPM2111

Items	Description
Genome size (bp)	3257744
Gene Total Length (bp)	2747361
Gene Length/Genome (%)	84.33
GC Content in Gene Region (%)	45.60
Intergenetic Region Length (bp)	510383
Intergenetic Length/Genome (%)	15.67
GC Content in Intergenetic Region (%)	38.76
Number of tRNAs	72
Number of 5S rRNAs	6
Number of 16S rRNAs	5
Number of 23S rRNAs	5
Number of sRNAs	41
Number of coding genes in the NR	3051
Number of coding genes in the Swiss-Prot	2156
Number of coding genes in the Pfam	2527
Number of coding genes in the COG	2403
Number of coding genes in the GO	2082
Number of coding genes in the KEGG	2138