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Running head: Probiotic characteristics of SKO-001 in *C. elegans* using multi-omics

Enhanced Longevity and Immunity in *Caenorhabditis elegans* through ingestion of *Lactiplantibacillus plantarum* SKO-001: A Multi-Omics Study

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ABSTRACT

Lactic acid bacteria are widely used as probiotics owing to their health-promoting properties. This study aimed to evaluate *Lactiplantibacillus plantarum* SKO-001 (SKO-001) as a probiotic candidate using *Caenorhabditis elegans* as a model organism. Our findings indicate that SKO-001 shows significantly stronger adhesive properties in *C. elegans* compared to *Escherichia coli* OP50, a standard dietary component used in laboratory settings for *C. elegans*, and the well-known probiotic *Lacticaseibacillus rhamnosus* GG (LGG). SKO-001 led to a significant increase in the longevity of *C. elegans* compared to those fed OP50. Additionally, pre-conditioning with SKO-001 significantly enhanced resistance to foodborne pathogenic bacteria. Transcriptomic analysis revealed that *C. elegans* fed with SKO-001 showed a significant increase in the expression of genes involved in the innate immune system, particularly those related to C-type lectins and lysozymes, compared to those fed with OP50. This suggests that feeding SKO-001 may boost immune responses against pathogens. Metabolomic analysis showed higher levels of lactic acid, L-valine, and L-isoleucine in *C. elegans* fed SKO-001 than in those fed OP50. Taken together, this research demonstrates the health-promoting potential of *Lactiplantibacillus plantarum* SKO-001 through multi-omics analysis, highlighting its capacity to extend lifespan and boost immune response in *C. elegans.*

Keywords: *Lactiplantibacillus plantarum*, *Caenorhabditis elegans*, longevity, immune response, multi-omics analysis

1. INTRODUCTION

Lactobacillus, a widely used probiotic, is recognized for its ability to extend lifespan, boost the immune system, and promote growth (Lee et al., 2022; Oh et al., 2023). Additionally, *Lactobacillus* species possess protective properties by preventing the invasion and colonization of pathogens and producing antipathogenic metabolites like lactic acid, hydrogen peroxide, bacteriocins, and phenylacetic acid (Chae et al., 2024; Cho et al., 2024; Eum et al., 2024; Han et al., 2024; Park et al., 2023b). They also provide immunological benefits by modulating the host's immune function (Dimitrijevic et al., 2014; Jaafar et al., 2024). Within this group, *Lactobacillus plantarum*, recently denominated as *Lactiplantibacillus plantarum* stands out as a promising probiotic (Kim et al., 2023a; Ryu et al., 2023; Song et al., 2023b; Yang et al., 2022). The safety of *Lactiplantibacillus plantarum* SKO-001, used in this study, has been confirmed in previous studies conducted on both mice and humans (Choi et al., 2023b; Shin et al., 2024).

Caenorhabditis elegans is an ideal surrogate animal model for studying microbe-host interactions (Kumar et al., 2020). *C. elegans* is extensively utilized as a model organism in research, owing to its numerous advantages, including a short lifespan, simple genetics, cost-effectiveness, ease of handling, and suitability for high-throughput screening (Choi et al., 2023a; Kang et al., 2024; Kim and Mylonakis, 2012; Lee et al., 2024b). Many research groups use *C. elegans* as a host model to evaluate the probiotic characteristics of various candidate strains (Kim et al., 2021; Kim and Mylonakis, 2012; Park et al., 2018). Bacteria commonly used as dietary resources for *C. elegans* can influence its phenotypes, including lifespan (Grompone et al., 2012; Kang et al.) and immune response (Kim and Mylonakis, 2012; Park et al., 2020). Administering bacteria directly to *C. elegans* allows researchers to study microbe-host interactions while minimizing the influence of external nutrients. This makes *C. elegans* a suitable model organism for studying the characteristics of probiotics and their effects on hosts.

Whole-transcriptome analysis is commonly used to explore changes in multiple genetic pathways in *C. elegans*. *C. elegans* is well-suited for investigating genetic pathways and observing the effects of

probiotics on aging and innate immunity (Kim et al., 2021; Lee et al., 2024b). Additionally, metabolomics has been used in *C. elegans* studies to identify metabolites linked to longevity and innate immunity (Lee et al., 2024a).

Various studies with *C. elegans* have shown that the consumption of specific *Lactobacillus* strains can enhance both lifespan and immune health (Kim et al., 2021; Lee et al., 2024a; Park et al., 2018). This study aimed to assess the probiotic properties of the candidate strain *Lactiplantibacillus plantarum* SKO-001 in *C. elegans* using multi-omics analysis

2. MATERIALS AND METHODS

2.1. Bacterial strains and culture conditions

In all experiments, the *C. elegans* strain *fer-15; fem-1*, which cannot produce progeny at 25 ℃, was used. *Escherichia coli* OP50 (OP50) was cultured in Luria-Bertani broth (BD Biosciences, Sparks, MD, USA) at 37 ℃ for 24 h. *Lacticaseibacillus rhamnosus* GG (LGG) and *Lactiplantibacillus plantarum* SKO-001 (Accession No. KCTC 14816BP) were grown in De Man-Rogosa-Sharpe broth (BD Biosciences, Sparks, MD, USA) at 37 ℃ for 48 h. SKO-001 was isolated from *Angelica gigas* Nakai and obtained from Kolmar BNH Co., Ltd. (Seoul, Korea). Four foodborne pathogenic bacteria were cultured as follows: *E. coli* O157:H7 EDL933 in Luria-Bertani broth at 37 ℃ for 24 h, *Salmonella* Typhimurium SL1344 in nutrient broth (BD Biosciences) at 37 ℃ for 24 h, and *Staphylococcus aureus* Newman and *Listeria monocytogenes* EGD-e in Brain Heart Infusion broth (BD Biosciences) at 37 ℃ for 24 h.

2.2. *In vivo* adhesive assay using *C. elegans*

To evaluate the colonization of SKO-001 in the *C. elegans* intestine, adhesion assays were conducted following established protocols (Lee et al., 2024a). *C. elegans* were cultured on NGM plates with OP50 until they contain eggs. Eggs were extracted using a sodium hypochlorite-sodium hydroxide solution, and synchronized L1 worms were grown on NGM plates with OP50 until the L4 stage at 25 ℃. Worms were then transferred to NGM plates seeded with OP50, SKO-001, or LGG (all at 8.0 × 10^9 colony-forming units [CFU/mL]). After 48 h, 10 worms from each group were placed on Brain Heart Infusion agar with gentamycin (25 μg/mL) for 5 min. Worms were transferred to 1.5-mL Eppendorf tube containing M9 buffer with Triton X-100, then mechanically disrupted. Samples were spread on Luria-Bertani agar for OP50 and De Man-Rogosa-Sharpe agar for LGG and SKO-001, incubated at 37 ℃ for 48 h. The experiment had 6 replicates per treatment, with 10 worms per replicate, totaling 60 worms per treatment group.

2.3. *C. elegans* life span and killing assay

To evaluate the impact of SKO-001 on *C. elegans* longevity and immune response to foodborne pathogens, slight modifications were made to previously established methods (Lee et al., 2024a; Park et al., 2018).

For lifespan assay, synchronized L1 stage worms were grown on NGM agar plates with OP50 until the L4 stage at 25 ℃. The worms were then individually transferred to 35-mm NGM agar plates seeded with OP50, SKO-001, or LGG (all at 8.0×10^9 CFU/mL). Each treatment group consisted of 90 worms, split across three plates (30 worms per plate), and kept at 25 ℃. Survival was recorded daily, and worms were moved to fresh plates every two days. Worms were assessed as alive or dead by gently touching them with a platinum wire. The experiment continued until all *C. elegans* in each group died.

For killing assay, L4 stage worms were transferred to 35-mm NGM agar plates seeded with OP50, SKO-001, or LGG (all at 8.0×10^9 CFU/mL) for a 48 h pre-conditioning period. Worms were then moved to NGM plates containing foodborne pathogens: *E. coli* O157:H7 EDL933, *S.* Typhimurium SL1344, *S. aureus* Newman, and *L. monocytogenes* EGD-e (all at 8.0×10^9 CFU/mL) and kept at 25 ℃. Each treatment group had 90 worms, divided across three plates (30 worms per plate). Worms were enumerated daily and moved to fresh plates every two days, with survival checked by gently touching them with a platinum wire. The experiment continued until all worms in each treatment group died.

2.4. Body size and locomotive activity

Locomotion and body dimensions were evaluated using Wormlab® software (MBF Bioscience, Vermont, USA), with slight modifications from the previous study (Shen et al., 2018). L4 stage C. elegans were exposed to OP50, SKO-001, or LGG (all at 8.0×10^9 CFU/mL) for 48 h, then moved to low-peptone NGM plates seeded with OP50. Filming began after a 10 min acclimation, with each video lasting 1 min for tracking analysis. Measurements included width, length, and peristaltic speed (μm/s). Ten worms per group were evaluated, and experiments were performed in triplicate.

The pharyngeal pumping rate, indicating food intake, was measured using a stereomicroscope by counting pharyngeal contractions over 30 sec. At least 10 worms per group were measured, with all experiments conducted in triplicate.

2.5. RNA isolation and transcriptomic analysis

Transcriptomic analysis was conducted with slight modifications (Ryu et al., 2021). L4 stage *C. elegans* were placed on NGM plates containing either OP50 (8.0×10^9 CFU/mL) or SKO-001 (8.0×10^9 CFU/mL). After a 48 h, total RNA was extracted using TRIZOL (Invitrogen, Carlsbad, CA, USA) and purified with the RNeasy Mini Kit (QIAGEN, Valencia, CA, USA). RNA-seq was performed using a TruSeq RNA Sample Prep Kit v2 (Illumina, San Diego, CA, USA) and sequenced on an Illumina NovaSeq 6000 platform with paired-end reads (2 × 150 bp). Trimmomatic 0.38 was used for quality trimming (Bolger et al., 2014). Reads shorter than 36 bp were discarded. Hisat2 v2.1.0 was used to create the reference genome index, and uniquely mapped reads were quantified using Subread/featureCounts v1.5.1. Genes with |log2-fold change| > 1 and *p*-value < 0.05 were considered significantly different. In this experiment, only genes with a significant difference (p-value < 0.05) and a fold change greater than 2 were included in the transcriptomic analysis. Functions of differentially expressed genes (DEGs) were identified using Database for Annotation, Visualization, and Integrated Discovery (DAVID), with network analysis performed using Cytoscape.

2.6. Metabolites extraction and metabolomic analysis

Metabolomic analysis was performed to evaluate metabolite variations using the previous method (Lee et al., 2024b). L4 stage worms were provided with either OP50 (8.0×10^9 CFU/mL) or SKO-001 (8.0×10^9 CFU/mL) for 48 h. The worms were rinsed six times with sterile deionized water, homogenized, combined with ice-cold methanol, vortexed, and centrifuged at 10,000 ×g for 10 min at 4 °C. The supernatants were then filtered through 0.2 μm syringe filters and vacuum dried.

For the gas chromatography-mass spectrometry (GC-MS) analysis, each sample was treated with 30 μL of methoxyamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) in pyridine (20 mg/mL)

and incubated at 30 ℃ for 90 min. Trimethylsilylation was then carried out by adding 50 µL of N,O-bis(trimethylsilyl)trifluoroacetamide (Sigma-Aldrich) and incubating at 60 ℃ for 30 min, followed by 10 µL of fluoranthene adding (Sigma-Aldrich).

GC-MS analysis used a TRACE™ 1310 Gas Chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) with an ISQ LT mass spectrometer (Thermo Fisher Scientific). Compounds were separated on a DB-5MS column (60 m × 0.25 mm, 0.25-µm film thickness, Agilent, Santa Clara, CA, USA). The temperature program started at 50 ℃ for 2 min, ramped to 180 ℃ at 5 ℃/min (held for 8 min), then increased to 325 ℃ at 2.5 ℃/min (held for 10 min). Samples were injected at 300 ℃ with helium as the carrier gas at 1.5 mL/min and a split ratio of 1:60. Detection used electron ionization at 70 eV and an ion source temperature of 270 ℃. The mass spectrometer scanned from 30 to 450 m/z at 5 spectra/sec. Metabolites were identified using the NIST Mass Spectral Library (version 2.0; NIST, Gaithersburg, MD, USA) and data analyzed with MetaboAnalyst 5.0 (Pang et al., 2021). Only metabolites with a match score above 850 in the NIST library were included in the metabolomic analysis for this study.

2.7. Statistics

The Kaplan–Meier method was used to analyze the lifespan and killing assay data for *C. elegans*, and results were visualized with SigmaPlot 12.0 (Systat Software Inc). Statistical analysis of other datasets was performed using Prism 9 (GraphPad Software, USA). Significance levels were set at *p*-values of <0.05 (*), 0.01 (**), 0.001 (***), and 0.0001 (****). Graphs are presented as mean ± standard error of the mean (SEM).

2.8. Data Availability

The manuscript contains all the data needed to support the study's conclusions and has been uploaded to the NCBI SRA database under Bioproject number PRJNA1132481.

3. RESULTS

3.1. Evaluation of *L. plantarum* SKO-001 adhesion ability in *C. elegans*

We employed *C. elegans* to investigate the *in vivo* adhesion properties of *Lactiplantibacillus plantarum* SKO-001 (SKO-001). *Escherichia coli* OP50 (OP50) served as a negative control, while *Lacticaseibacillus rhamnosus* GG (LGG) was used as a positive control. The adhesion assessment was performed following 48 h of exposure to OP50, SKO-001, or LGG. Our findings revealed that SKO-001 exhibited a significantly greater adhesion capability compared to both OP50 and LGG (p <0.0001 for each comparison with OP50 and LGG) (Fig. 1). This suggests that SKO-001 shows remarkable adhesive properties in *C. elegans* in comparison to OP50 and LGG.

3.2. Analysis of the influence of *L. plantarum* SKO-001 on longevity and immune response in *C. elegans*

We explored the impact of SKO-001 on the lifespan of *C. elegans* by treating the worms with OP50, SKO-001, or LGG. The group receiving OP50 was labeled as the OP50 group, the group receiving SKO-001 was labeled as the SKO-001 group, and the group receiving LGG was labeled as the LGG group. *C. elegans* in the SKO-001 group had a significantly longer lifespan compared to those in the OP50 group (p = 0.0000) (Fig. 2a). Moreover, no significant difference in lifespan was observed between the SKO-001 and LGG groups (p = 0.1506) (Fig. 2a). These findings suggest that SKO-001 treatment significantly prolonged the lifespan of *C. elegans*.

We next performed killing assays to assess whether SKO-001 improved the ability of *C. elegans* to defend against various foodborne pathogenic bacteria. After a 48 h pre-conditioning period with OP50, SKO-001, or LGG, the worms were placed on NGM plates seeded with foodborne pathogens. *E. coli* O157:H7 EDL933 and S. Typhimurium SL1344, both gram-negative bacteria, and *S. aureus* Newman and *L. monocytogenes* EGD-e, both gram-positive bacteria, were used. *C. elegans* that had been pre-conditioned with SKO-001 showed a significantly reduced susceptibility to the gram-negative bacteria compared to those pre-conditioned with OP50 (p = 0.0003 for *E. coli* O157:H7

EDL933 and p = 0.0000 for *S.* Typhimurium SL1344) (Fig. 2b and 2c). However, no significant differences in susceptibility were observed between *C. elegans* pre-conditioned with SKO-001 and those pre-conditioned with LGG (p = 0.6531 for *E. coli* O157:H7 EDL933 and p = 0.8388 for *S.* Typhimurium SL1344) (Fig. 2b and 2c). In experiments involving gram-positive bacteria, *C. elegans* pre-conditioned with SKO-001 showed significantly better survival rates compared to those pre-conditioned with OP50 (p = 0.0000 for *S. aureus* Newman and p = 0.0000 for *L. monocytogenes* EGD-e) (Fig. 2d and 2e). No notable difference in survival was observed between *C. elegans* pre-conditioned with SKO-001 and those pre-conditioned with LGG (p = 0.1506 for *S. aureus* Newman and p = 0.3670 for *L. monocytogenes* EGD-e) (Fig. 2d and 2e). Overall, these findings suggest that pre-conditioning with SKO-001 improves the resistance of *C. elegans* to infections caused by both gram-negative and gram-positive pathogenic bacteria.

3.3. Evaluation of the impact of *L. plantarum* SKO-001 on *C. elegans* phenotype

To evaluate the impact of SKO-001 on *C. elegans*' phenotype, we assessed body size and locomotive activity. Worms fed SKO-001 exhibited significantly larger body dimensions, including both length and width, compared to those fed OP50 (p <0.0001 for both length and width) and LGG (p <0.0001 for both length and width) (Fig. 3a and 3b). However, no significant difference in peristaltic speed, a measure of worm activity, was observed between the SKO-001 and OP50 groups (p = 0.7777) (Fig. 3c). Similarly, the peristaltic speed was comparable between the SKO-001 and LGG groups (p = 0.9783) (Fig. 3c). In the pumping rate assay, which reflects food intake, worms in the SKO-001 group showed a significant increase compared to those in the OP50 and LGG groups (p <0.0001 for OP50 and p = 0.0015 for LGG) (Fig. 3d). Overall, these findings suggest that SKO-001 enhances both body size and pumping rate in *C. elegans*.

3.4. Transcriptomic analysis of *C. elegans* after exposure to *L. plantarum* SKO-001

A transcriptomic analysis was conducted to investigate the gene expression alterations in *C. elegans* induced by SKO-001 feeding in comparison to OP50. Genes showing more than a 2-fold increase in

252 expression with SKO-001 were identified and examined using DAVID to determine associated
253 upregulated pathways. The top 10 pathways related to these significantly upregulated genes are
254 detailed in Table 1. Consistent with the findings from the killing assays, pathways related to the innate
255 immune response and defense mechanisms against both gram-positive and gram-negative bacteria
256 were notably upregulated in SKO-001-fed *C. elegans*. Specifically, genes linked to C-type lectins
257 (clec-41, clec-66, clec-86, clec-186, and clec-187) and lysozymes (lys-1, lys-2, lys-3, lys-7, and lys-8)
258 were significantly upregulated in response to SKO-001 (Table 2). To identify the Kyoto Encyclopedia
259 of Genes and Genomes pathways upregulated by feeding SKO-001, Cytoscape was performed with
260 genes that exhibited more than a 2-fold increase in *C. elegans* fed SKO-001 compared to those fed
261 OP50. The results identified several pathways that were significantly upregulated with SKO-001,
262 including those involved in drug metabolism, tryptophan metabolism, lysosomes, glycine, serine, and
263 threonine metabolism, sphingolipid metabolism, glycerophospholipid metabolism, longevity-
264 regulating pathways, and arginine and proline metabolism (Fig. 4). These results collectively indicate
265 that SKO-001 enhances immune response and promotes longevity.
266
267 **3.5. Metabolomic analysis of *C. elegans* after exposure to *L. plantarum* SKO-001**
268 Metabolomic analysis was conducted to evaluate the effect of SKO-001 on the metabolite
269 composition of *C. elegans*. The partial least squares-discriminant analysis revealed distinct clustering
270 of metabolite profiles between *C. elegans* fed SKO-001 and those fed OP50 (Fig. 5a). A heatmap of
271 the top 12 most significantly altered metabolites revealed increased levels of carbamic acid, lactic
272 acid, L-valine, and L-isoleucine in *C. elegans* receiving SKO-001, compared to the OP50 group (Fig.
273 5b). Quantitative analysis highlighted that metabolites such as lactic acid, succinic acid, L-aspartic
274 acid, and 3-oxaoct-4-en-2-imine were elevated by more than 2-fold in SKO-001 fed *C. elegans* (Fig.
275 5c). Additionally, a volcano plot showed that seven metabolites lactic acid, carbamic acid, L-
276 isoleucine, L-valine, 3-oxaoct-4-en-2-imine, nonanoic acid, and L-aspartic acid were significantly
277 upregulated in the SKO-001 group compared to the OP50 group (Fig. 5d). Overall, these findings

indicate that SKO-001 alters the metabolite profile of *C. elegans*, with a notable increase in several key metabolites, including lactic acid.

4. DISCUSSION

Lactic acid bacteria, especially those belonging to the *Lactobacillus* genus, are well-known for their beneficial effects on health and are frequently used as probiotics. *Lactobacillus plantarum* has been reported to positively influence longevity and immune responses in various studies (Kim et al., 2022; Kumar et al., 2022; Oh et al., 2022b). Therefore, we investigated the potential of *L. plantarum* SKO-001 (SKO-001) as a probiotic candidate using *C. elegans*.

Previous studies have demonstrated that the ability of probiotic bacteria to adhere to the host's gastrointestinal tract is a key criterion in their selection. This adhesive ability facilitates colonization and enhances immunomodulatory effects by stimulating the gut barrier and metabolic function (Kim et al., 2023b; Kim et al., 2023c; Song et al., 2023a). Consequently, probiotics can survive, proliferate, and deliver numerous health benefits to their host (KINARA et al., 2024; Oh et al., 2022a; Park et al., 2023a; Park et al., 2024). In our study, SKO-001 demonstrated significantly higher adhesive ability than OP50 and LGG. Lifespan measurements are extensively used to study aging processes. *C. elegans*, with its short lifespan, is a suitable *in vivo* model for measuring the ability of candidate probiotic bacteria (Tissenbaum, 2015). In the lifespan assay, SKO-001 significantly increased the lifespan of *C. elegans* compared to OP50, showing no significant difference from LGG. This result supports earlier findings that *Lactobacillus* species with probiotic properties can increase the lifespan of *C. elegans* (Heo et al., 2018; Lee et al., 2024a). Similarly, the killing assay revealed that pre-conditioning with SKO-001 significantly improved the immune response of *C. elegans* against both gram-negative and gram-positive pathogenic bacteria. This observation aligns with previous research, which underscores the strong antimicrobial properties of *L. plantarum* against pathogens and its capacity to boost the immune response in *C. elegans* (Li et al., 2017; Mun et al., 2019).

The quality of food affects worm phenotypes (Shtonda and Avery, 2006). Additionally, different bacteria can impact the growth of C. elegans to varying degrees (Avery and You, 2018). Therefore, we measured worm size and locomotor activity to assess the quality of SKO-001 as a food source and to determine whether it could alter the phenotype of the worms. Worms fed SKO-001 exhibited a

significant increase in both length and width compared to those fed OP50 and LGG. Furthermore, SKO-001 also improved the pumping rate more effectively than OP50 and LGG. These results indicate that SKO-001 not only caused notable phenotypic changes in the worms but also enhanced their growth performance.

Our study indicates that pre-conditioning *C. elegans* with SKO-001 enhances its immune defense against foodborne pathogens. We hypothesized that pre-conditioning upregulates specific immune-related genes. Transcriptomic analysis revealed that genes with more than a 2-fold increase in *C. elegans* fed SKO-001, compared to those fed OP50, were predominantly associated with innate immunity.

Notably, genes related to C-type lectins (*clec-41*, *clec-66*, *clec-86*, *clec-186*, and *clec-187*) and lysozymes (*lys-1*, *lys-2*, *lys-3*, *lys-7*, and *lys-8*) showed significant upregulation following SKO-001 treatment. In *C. elegans*, *clec* genes encode a variety of proteins with C-type lectin-like domains (CTLDs), which play a role in pathogen defense (Schulenburg et al., 2008). Previous studies have shown that *clec-41* plays a vital role in the resistance to the gram-positive pathogen *Bacillus thuringiensis* MYBt18247 (Pees et al., 2021). Similarly, *clec-86* has been demonstrated to be essential for defense against the gram-positive pathogen *Microbacterium nematophilum (O'Rourke et al., 2006).* These results suggested that *clec-41 and clec-86 play a crucial role in resistance against pathogenic bacteria. Consistent with prior studies, the expression levels of both clec-41 and clec-86 were notably higher in C. elegans fed SKO-001 compared to those fed OP50. Lysozymes function as antimicrobial agents within the C. elegans gut, breaking down bacterial cells (Ciancio, 2016).* Genes related to lysozymes, including *lys-1, lys-3, and lys-7,* are essential for the defense mechanisms in *C. elegans* (Schulenburg et al., 2008). We found that these genes were significantly upregulated following SKO-001 feeding. This suggests that the enhanced expression of both C-type lectin and lysozyme-related genes induced by SKO-001 contributes to a more robust immune response against pathogenic bacteria.

In the metabolomic analysis, *C. elegans* fed SKO-001 showed a notable increase in lactic acid compared to those fed OP50. Lactic acid, often produced by lactic acid bacteria, is associated with

enhanced defense and resistance in *C. elegans* (Fernández et al., 2003). The higher levels of lactic acid observed with SKO-001 treatment likely contributed to an improved immune response against pathogens. Additionally, the branched-chain amino acids L-isoleucine and L-valine, which were elevated in SKO-001 fed *C. elegans*, are crucial for various physiological processes. Previous studies have demonstrated that supplementing *C. elegans* with L-valine and L-isoleucine can significantly prolong their lifespan (Wang and Zhang, 2018; Wang et al., 2018). Collectively, the increased levels of metabolites observed with SKO-001 feeding may have contributed to enhanced longevity and improved immune response in *C. elegans*.

5. CONCLUSION

In conclusion, we investigated the probiotic potential of *L. plantarum* SKO-001 (SKO-001) using *C. elegans* as a model organism. SKO-001 showed superior adhesion capabilities compared to OP50 and LGG, suggesting its effectiveness in gastrointestinal colonization. Additionally, SKO-001 significantly prolonged the lifespan of *C. elegans*, improved its resistance to foodborne pathogens, and supported its growth. Transcriptomic analysis revealed notable upregulation of genes related to the innate immune system, particularly those involved in C-type lectins and lysozymes. Metabolomic analysis showed increased levels of lactic acid, L-valine, and L-isoleucine in *C. elegans* treated with SKO-001. Overall, our findings suggest that *L. plantarum* SKO-001 is a promising probiotic with potential benefits for improving longevity and boosting immune function.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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535 **Table 1. Transcriptomic analysis of *Caenorhabditis elegans* fed with *Lactiplantibacillus***

536 ***plantarum* SKO-001**

Term[a]	Gene count	%	*p* value
Innate immune response	75	8.4	0.000
Defense response to gram-positive bacteria	20	2.2	0.000
Anatomical structure development	23	2.6	0.000
Peptidoglycan catabolic process	6	0.7	0.000
Cell wall macromolecule catabolic process	6	0.7	0.000
Defense response to gram-negative bacteria	12	1.3	0.000
Glutathione metabolic process	10	1.1	0.000
Proteolysis	26	2.9	0.002
Lipid metabolic process	19	2.1	0.003
Lipid transport	7	0.8	0.003

537 **[a]** The top 10 pathways associated with genes that are significantly upregulated by >2.0 folds in *C.*

538 *elegans* after 48 h of exposure to *L. plantarum* SKO-001 compared to *Escherichia coli* OP50

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Table 2. Transcriptomic analysis of *Caenorhabditis elegans* fed with *Lactiplantibacillus plantarum* SKO-001

Group and gene[a]	Gene number	Fold change	*p* value	Description
C-type lectin-related				
clec-41	CELE_B0365.6	5.315288	0.000	C-type lectin
clec-66	CELE_F35C5.9	2.534151	0.000	C-type lectin
clec-86	CELE_C54D1.2	6.759153	0.000	C-type lectin
clec-186	CELE_ZK896.7	5.362350	0.000	C-type lectin
clec-187	CELE_ZK896.6	4.920694	0.000	C-type lectin
Lysozyme-related				
lys-1	CELE_Y22F5A.4	2.592985	0.000	Lysozyme
lys-2	CELE_Y22F5A.5	6.513251	0.000	Lysozyme
lys-3	CELE_Y22F5A.6	2.661261	0.000	Lysozyme
lys-7	CELE_C02A12.4	3.604976	0.000	Lysozyme
lys-8	CELE_C17G10.5	2.087275	0.000	Lysozyme

[a] The list of genes associated with the innate immune response pathway that are significantly upregulated by >2.0 folds in *C. elegans* after 48 h of exposure to *L. plantarum* SKO-001 compared to *Escherichia coli* OP50

Figure legends

Fig. 1. Adhesion ability of *Lactiplantibacillus plantarum* SKO-001 in *Caenorhabditis elegans*

Adhesion ability of OP50, SKO-001, or LGG in *C. elegans* strain *fer-15; fem-1* after a 48 h exposure period. OP50, *Escherichia coli* OP50; SKO-001, *L. plantarum* SKO-001; LGG, *Lacticaseibacillus rhamnosus* GG. Statistical analysis is conducted using a one-way analysis of variance, and statistical significance is considered when p values are <0.05 (*), <0.01 (**), <0.001 (***), and <0.0001 (****). Statistical comparisons with SKO-001: p <0.0001 for both OP50 and LGG. Data are expressed as means ± *SEM*

Fig. 2. Lifespan and killing assay of *Caenorhabditis elegans* fed with *Lactiplantibacillus plantarum* SKO-001

Lifespan of *C. elegans* strain *fer-15; fem-1* fed OP50, SKO-001, and LGG. For the killing assay, *C. elegans* strain *fer-15; fem-1* was pre-conditioned with OP50, SKO-001, or LGG for 48 h and then infected with foodborne pathogenic bacteria (two gram-negative and two gram-positive bacteria). (A) Lifespan assay of *C. elegans* (B) Killing assay using *Escherichia coli* O157:H7 EDL933 cells (C) Killing assay using *Salmonella typhimurium* SL1344 (D) Killing assay using *Staphylococcus aureus* Newman (E) Killing assay using *Listeria monocytogenes* EGD-e. OP50, *E. coli* OP50; SKO-001, *L. plantarum* SKO-001; LGG, *Lacticaseibacillus rhamnosus* GG. Statistical analysis is conducted using Kaplan–Meier method, and differences are considered significant when the p value is <0.05 (*) and <0.01 (**) compared to OP50. Survival statistics in the lifespan assay compared to SKO-001: $p = 0.0000$ and $p = 0.1506$ for OP50 and LGG, respectively. Survival statistics for the killing assay compared to SKO-001: *E. coli* O157:H7 EDL933, $p = 0.0003$ and $p = 0.6531$ for OP50 and LGG, respectively; *S. typhimurium* SL1344, $p = 0.0000$ and $p = 0.8388$ for OP50 and LGG, respectively; *S. aureus* Newman, $p = 0.0000$ and $p = 0.1506$ for OP50 and LGG, respectively; *L. monocytogenes* EGD-e, $p = 0.0000$ and $p = 0.3670$ for OP50 and LGG, respectively.

Fig. 3. Body size and locomotive activity of *Caenorhabditis elegans* fed with *Lactiplantibacillus plantarum* SKO-001

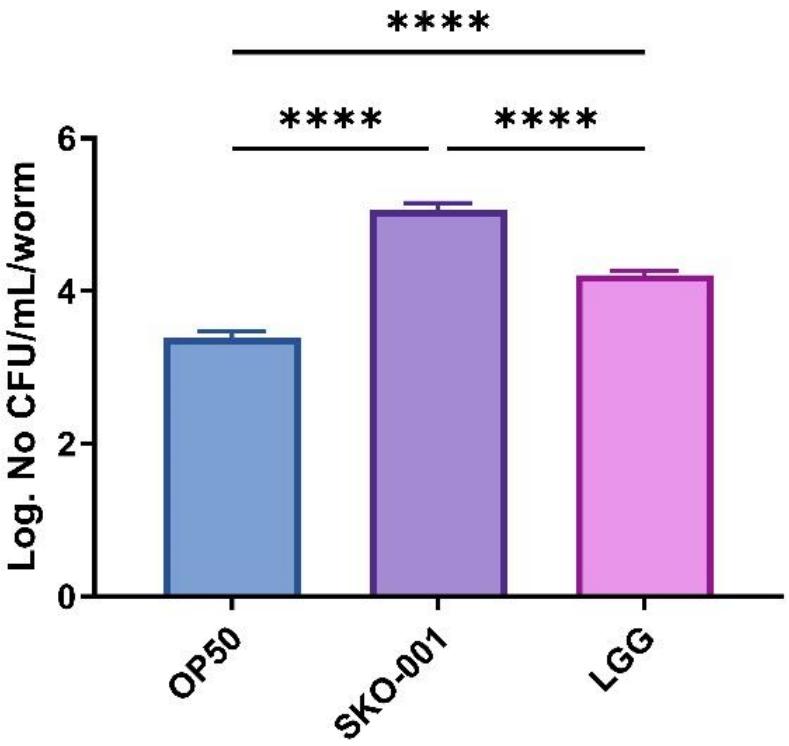
Body size and locomotive activity of *C. elegans* strains *fer-15; fem-1* after a 48-h exposure period with OP50, SKO-001, or LGG (A) length, (B) width, (C) peristaltic speed, and (D) pumping rate. OP50, *Escherichia coli* OP50; SKO-001, *L. plantarum* SKO-001; LGG, *Lacticaseibacillus rhamnosus* GG. Statistical analysis is conducted using a one-way analysis of variance, and statistical significance is considered when p values are <0.05 (*), <0.01 (**), <0.001 (***), and <0.0001 (****). Statistics compared to SKO-001: length, p <0.0001 and p <0.0001 for OP50 and LGG, respectively; width, p <0.0001 and p <0.0001 for OP50 and LGG, respectively; peristaltic speed, p = 0.7777 and p = 0.9783 for OP50 and LGG, respectively; pumping rate, p <0.0001 and p = 0.0015 for OP50 and LGG, respectively. Data are expressed as means ± *SEM*

Fig. 4. Transcriptomic analysis of *Caenorhabditis elegans* fed with *Lactiplantibacillus plantarum* SKO-001

The identification of Kyoto Encyclopedia of Genes and Genomes pathways related to genes is significantly upregulated by >2.0 folds in *C. elegans* after 48 h of exposure to *L. plantarum* SKO-001 as compared to *E. coli* OP50. Cytoscape is used for the analysis

Fig. 5. Metabolomic analysis of *Caenorhabditis elegans* fed with *Lactiplantibacillus plantarum* SKO-001

Comparison of the metabolite composition of *C. elegans* after 48 h of exposure to *L. plantarum* SKO-001 and *Escherichia coli* OP50 (A) PLS-DA (B) Volcano plot (C) The top 12 enriched heat maps (D) Quantitative graph depicting metabolites that changes by >2.0 folds

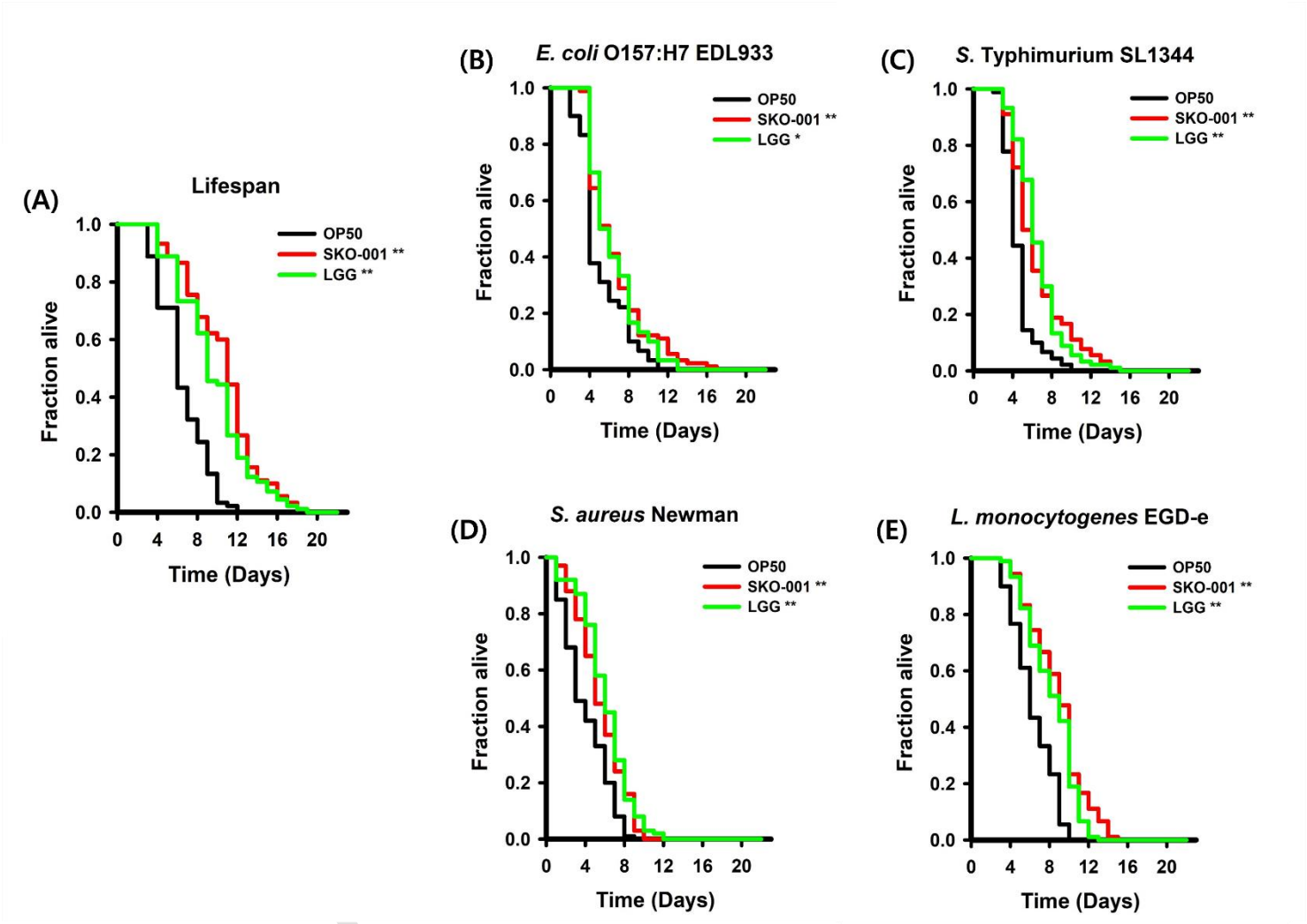


601

602 **Fig. 1.**

603

604


(A)
Lifespan
Fraction alive
Time (Days)
OP50
SKO-001 **
LGG **
(B)
E. coli O157:H7 EDL933
OP50
SKO-001 **
LGG *
(C)
S. Typhimurium SL1344
OP50
SKO-001 **
LGG **
(D)
S. aureus Newman
OP50
SKO-001 **
LGG **
(E)
L. monocytogenes EGD-e
OP50
SKO-001 **
LGG **

605

606 **Fig. 2**

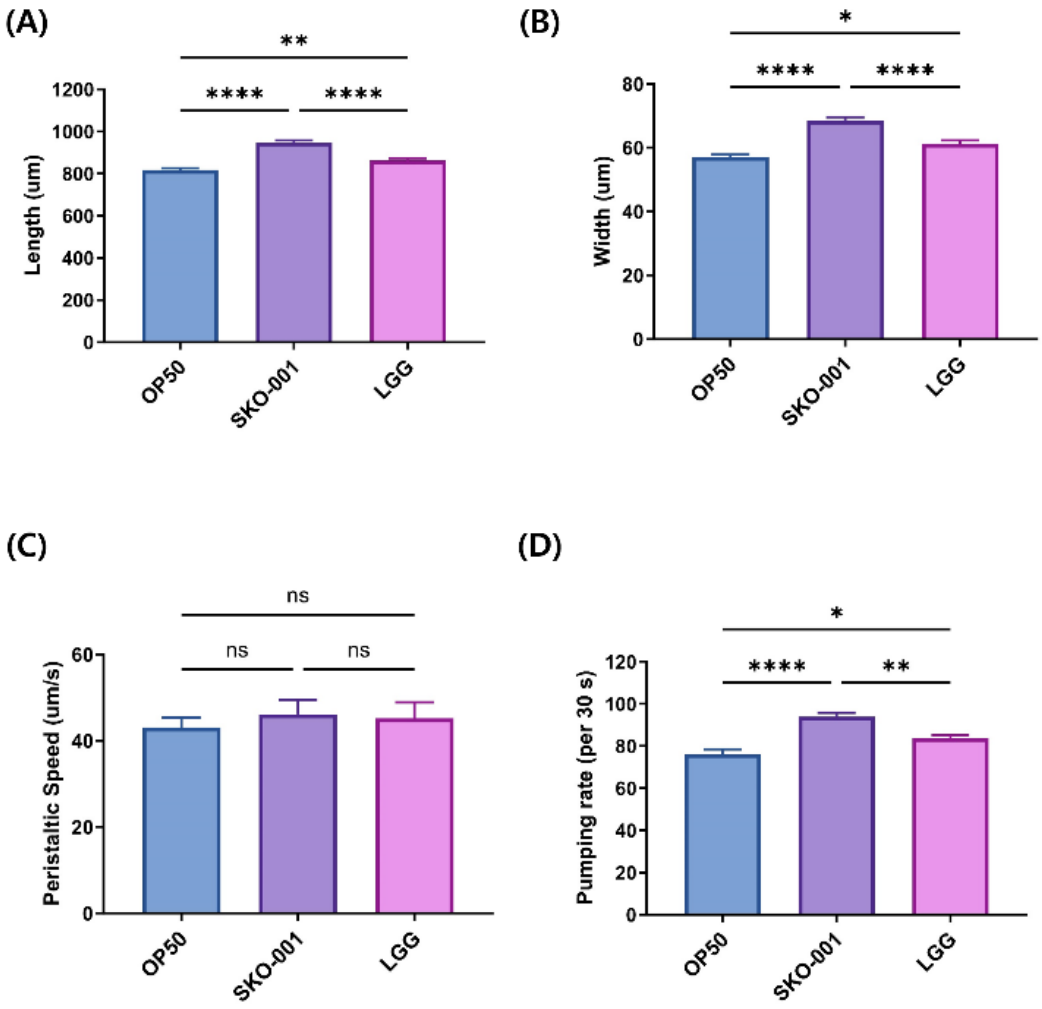
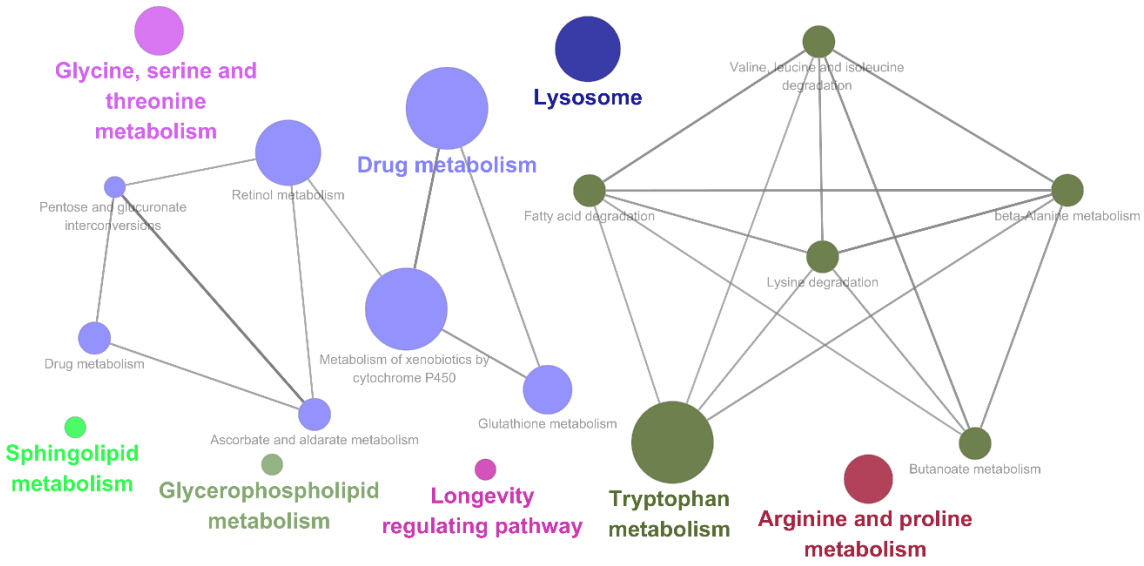

(A)
**
****
****
Length (um)
1200
1000
800
600
400
200
0
OP50
SKO-001
LGG
(B)
*
****
****
Width (um)
80
60
40
20
0
OP50
SKO-001
LGG
(C)
ns
ns
ns
Peristaltic Speed (um/s)
60
40
20
0
OP50
SKO-001
LGG
(D)
*
****
**
Pumping rate (per 30 s)
120
100
80
60
40
20
0
OP50
SKO-001
LGG

Fig. 3.



614

615 **Fig. 4.**

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617

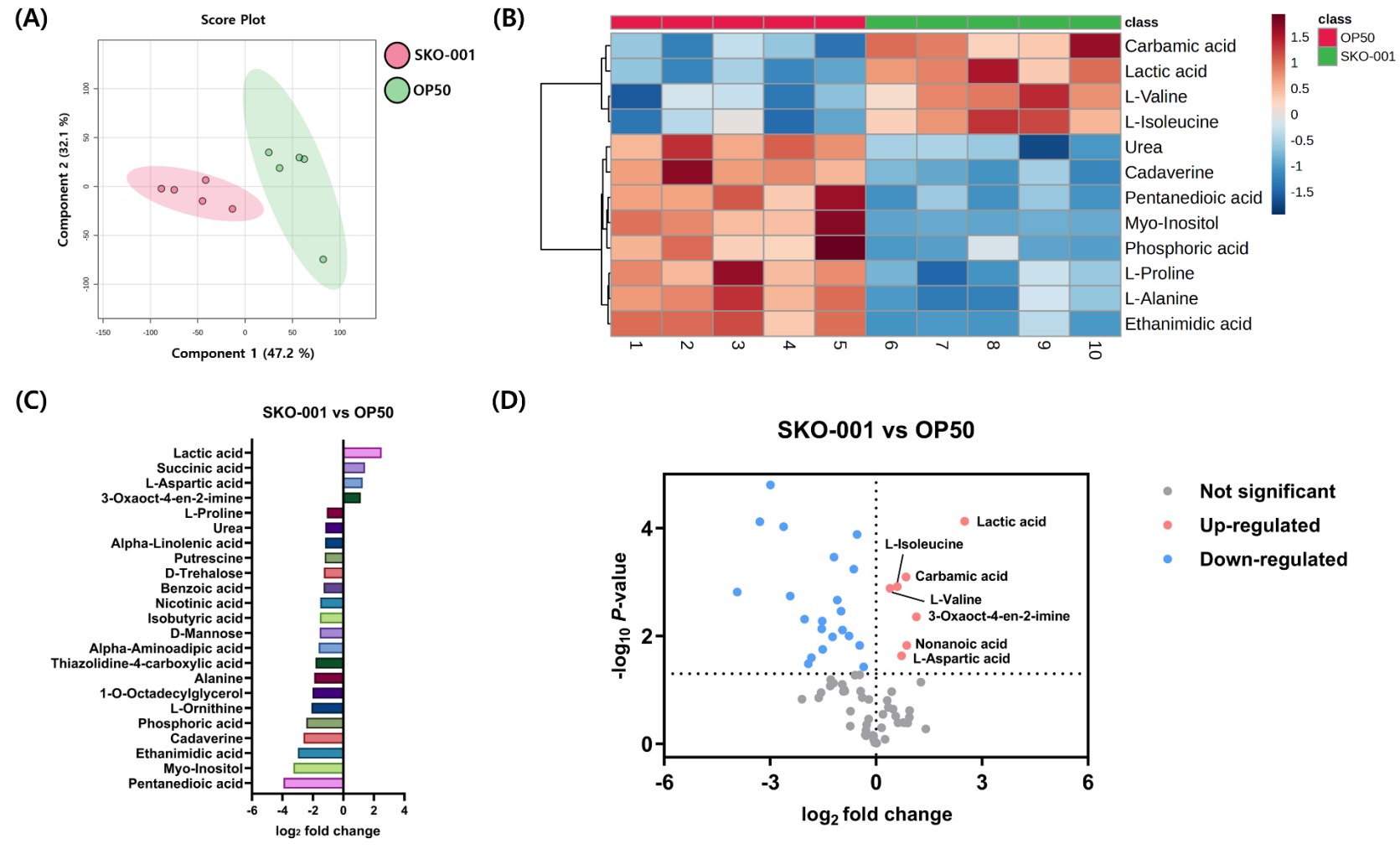
(A)
Score Plot
SKO-001
OP50
Component 2 (32.1 %)
Component 1 (47.2 %)
(B)
class
Carbamic acid
Lactic acid
L-Valine
L-Isoleucine
Urea
Cadaverine
Pentanedioic acid
Myo-Inositol
Phosphoric acid
L-Proline
L-Alanine
Ethanimidic acid
(C)
SKO-001 vs OP50
Lactic acid
Succinic acid
L-Aspartic acid
3-Oxaoct-4-en-2-imine
L-Proline
Urea
Alpha-Linolenic acid
Putrescine
D-Trehalose
Benzoic acid
Nicotinic acid
Isobutyric acid
D-Mannose
Alpha-Aminoadipic acid
Thiazolidine-4-carboxylic acid
Alanine
1-O-Octadecylglycerol
L-Ornithine
Phosphoric acid
Cadaverine
Ethanimidic acid
Myo-Inositol
Pentanedioic acid
log2 fold change
(D)
SKO-001 vs OP50
Lactic acid
L-Isoleucine
Carbamic acid
L-Valine
3-Oxaoct-4-en-2-imine
Nonanoic acid
L-Aspartic acid
-log10 P-value
log2 fold change
Not significant
Up-regulated
Down-regulated

Fig. 5