High-fat diet-induced oxidative stress links the increased colonization of *Lactobacillus sakei* in obesity population

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<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

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Obesity is a major public health problem related to various chronic health conditions. *Lactobacillus* species has been reported in obese individuals; however, its role is unknown. We compared the abundance and composition of *Lactobacillus* species by analyzing feces from 64 healthy control subjects and 88 obese subjects. We isolated one *Lactobacillus* strain from the feces of a subject with obesity and further analyzed its genetic and molecular features.

We found that an increased abundance of *Lactobacillus* sakei distinguished the fecal microbiota of the obese group from that of healthy subjects and that it was related to the increased levels of reactive oxygen species (ROS) induced by higher fat intake. The *L. sakei* ob4.1 strain, isolated from the feces of a subject with obesity, showed high catalase activity, which was regulated by oxidative stress at the gene transcription level. *L. sakei* ob4.1 maintained colon epithelial cell–adhesion ability under ROS stimulation, and treatment with saturated fatty acid increased colon epithelial ROS levels in a dose-dependent manner; however, *L. sakei* ob4.1 did not change the level of fat-induced colon epithelial ROS. Exposing mice to a high-fat diet revealed that high-fat diet–induced colon ROS was associated with the increased colonization of *L. sakei* ob4.1 through catalase activity. Four-week supplementation with this strain in mice fed a high-fat diet did not change their body weights or ROS levels.

A high-fat diet induces changes in the colon environment by increasing ROS levels, which provides a colonization benefit to an *L. sakei* strain with high catalase activity.

Key words : *Lactobacillus sakei*; obesity; gut microbiome; reactive oxygen stress; inflammation; high fat diet

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I. INTRODUCTION

Obesity is a multifactorial disease that involves both genetic and environmental factors[1,2], hence necessitating the identification of its risk and preventive factors to reduce the possibility of obesity and obesity-related complications.

To date, the relationship between gut microbiota and obesity has been widely studied [3,4]. The gut microbiota is known to cause obesity by various mechanisms including modulation of host genes that affect the extraction of energy from the diet[5]. On the other hand, changes in the gut environment induced by obesity can cause gut dysbiosis. The consumption of a high-fat diet, one of the main causes of obesity, is considered to play a key role in changing the gut environment. A high-fat diet increases the levels of oxidative stress in the colon [6,7] and affects the composition of the colonic microbiota [8,9]. Hence, a reciprocal relationship exists between obesity and the gut microbiota.

Although the causal relationship is not yet understood, obesity has been associated with specific groups of gut bacteria including *Lactobacillus*. The increased abundance of commensal *Lactobacillus* [10], particularly *Lactobacillus sakei* [11], in obese populations has been reported in case-control studies. However, many strains of *Lactobacillus* are used as probiotics and some strains of *Lactobacillus* have shown beneficial effects on obesity and obesity-related complications [12-14] in clinical trials. There is no evidence that the consumption of *Lactobacillus* as probiotics can induce obesity in a healthy population[15] and the mechanism underlying the relationship between an increase in

Lactobacillus and obesity is still unknown and likely to vary across strains.

Lactobacillus has been reported to show high diversity in its resistance to oxidative stress across species [16,17] and strains [18], based on catalase activity. For example, *L. sakei,* one of the *Lactobacillus* strains related with obesity, is highly resistant to oxidative stress via catalase activity [19,20] with a high degree of intraspecies diversity regarding the response to oxidative stress [18]. Hence, it may be hypothesised that the colonisation of different *Lactobacillus* strains can be determined by the oxidative stress levels in the colon, induced by a high-fat diet in obesity. The first purpose of this study was comparing the abundance and patterns of commensal *Lactobacillus* in obese and healthy subjects. Second, we investigated the role of high-fat diet induced ROS in the relationship between *Lactobacillus* species and obesity. Third, we isolated a strain of a *Lactobacillus* species related to obesity to further investigating its genetic and molecular characteristics as they relate to colonic oxidative stress in obesity. Last, we performed *in vivo* experiment to confirm the causal relationship between high-fat induced ROS and the colonization of obesity-related *Lactobacillus* species using high-fat diet-induced obesity mice.

II. MATERIALS AND METHODS

1. Study participants

All subjects participated in the study voluntarily, and written informed consent was obtained from each subject. The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of Yonsei University College of Medicine and CHA Bundang Medical Centre. Participants were recruited from among the individuals that visited the hospital (Department of Family Medicine of Severance Hospital and Chaum Hospital) for either a regular health check-up or to reduce their body weight. Obese subjects were defined as those having a body mass index (BMI) > 25 kg/m^2, and control subjects were healthy participants over 19 years of age with a BMI between 19 and 23 kg/m^2. We excluded individuals with a history of any type of

cancer, inflammatory bowel disease, or abdominal surgery. Subjects with a history of taking probiotics or antibiotics, within 4 weeks before participation, were also excluded. Overall, 64 healthy control subjects and 88 obese subjects were finally included.

2. Questionnaire

All participants completed a questionnaire about lifestyle factors, such as physical activity, smoking, and alcohol consumption. Regular exercise was defined as activity more than a moderate degree (walking more than 5 times/week for 30 min, moderate-intensity physical activity more than 5 times/week for 30 min, or vigorous-intensity physical activity more than 3 times/week for 20 min) in recent 1 week. Smoking was defined as being a current smoker and alcohol drinking was defined as drinking alcoholic beverage ≥ 70 g/day, or more frequent than once a week. A 24-h dietary recall was conducted with each participant for nutrition assessment. A standard form included information about each food item consumed (amount, ingredients, and number of times within the previous 24 h). Nutritional analysis, including total calorie intake, total fat intake, and fatty acid intake, was performed using CAN-PRO software 5.0 (Korean National Society, Seoul, Korea), and each value was adjusted by calculating nutrient density and expressing it as total amount/1000 kcal.

3. Stool sample collection

Participants were provided sterile plain tubes, with no chemical additive, for stool collection. Fresh stool samples (approximately 2 g) were collected and transported to the laboratory within 1 hour; 100 mg of fresh stool was used for the *Lactobacillus* culture study, and the rest was stored at −80 °C until subsequent analysis.

4. Human faecal ROS measurement

Approximately 100 mg of faeces was homogenised in a phosphate-buffered solution (PBS) and centrifuged at 3,000 rpm at 4 °C for 20 min. The supernatant was used for analysis. The human faecal ROS level was measured using a commercial enzyme-

linked immunosorbent assay kit (Human ROS ELISA kit (MyBioSource INC., CA, U.S.A)), according to the manufacturer's instructions.

5.16S rRNA quantitative polymerase chain reaction (PCR) analysis of total *Lactobacillus*

Genomic DNA was extracted from the faeces using a QIAmp DNA Stool Mini Kit (Qiagen, CA, U S A), according to the manufacturer's protocol. The relative abundance of *Lactobacillus* was analysed via quantitative real-time PCR according to the method described by Yoon et al. [21]. Briefly, 2 μL of 10-fold-diluted genomic DNA, extracted from the faeces, was taken as the template; SYBR Green PCR master mix (Applied Biosystems, CA, U S A) and appropriate primer sets designed to amplify the 16S region [22] (Table 1) were used to perform quantitative real-time PCR. The transcript levels of target genes were normalised to the gene expression levels of the housekeeping gene *gapdh*.

6. Polymerase chain reaction (PCR) analysis of *Lactobacillus* species

Genomic DNA was extracted from the faeces samples using a QIAmp DNA Stool Mini Kit (Qiagen, CA, U S A), according to the manufacturer's protocol. The *Lactobacillus* species-specific PCR was performed using primers (Table 1) [23-26], targeted on the 16S–23S ribosomal RNA intergenic spacer region. The reaction mixture comprised 100 ng of bacterial DNA from the faeces, PCR Supermix High Fidelity (Invitrogen, CA, U S A). Amplified PCR products were detected via agarose gel electrophoresis using a 1.5% agarose gel, with ethidium bromide staining and UV transillumination.

Table 1. List of primers used in the study

Organism	Target gene	Sequence (5'-3')
Genus Lactobacillus	16s rRNA	F:AGCAGTAGGGAATCTTCCA
		R: CACCGCTACACATGGAG

L.sakei	16s-23s rRNA	F: GAGCTAATCCCCCATAATGAAACTAT
		R: GATAAGCGTGAGGTCGATGGTT
	katA	F: AATTGCCTTCTTCCGTGTA,
		R: AGTTGCGCACAATTATTTTC
L.paracasei	16s-23s rRNA	F: ACATCAGTGTATTGCTTGTCAGTGAATAC
		R: CCTGCGGGTACTGAGATGTTTC
L.casei	16s-23s rRNA	F: CTTCCTGCGGGTACTGAGATGT
		R: CTATAAGTAAGCTTTGATCCGGAGATTT
L.rhamnosus	16s-23s rRNA	F: CGGCTGGATCACCTCCTTT
		R: GCTTGAGGGTAATCCCCTCAA
L.gasseri	16s-23s rRNA	F: AGCGACCGAGAAGAGAGAGA
		R: TGCTATCGCTTCAAGTGCTT
L.salivarius	16s-23s rRNA	F: GTCGTAACAAGGTAGCCGTAGGA
		R: TAAACAAAGTATTCGATAAATGTACAGGTT
L.plantarum	16s-23s rRNA	F: TGGATCACCTCCTTTCTAAGGAAT
		R: TGTTCTCGGTTTCATTATGAAAAAATA
L.reuteri	16s-23s rRNA	F: ACCGAGAACACCGCGTTATTT
		R: CATAACTTAACCTAAACAATCAAAGATTGTCT
L.fermentum	16s-23s rRNA	F: ACTTAACCTTACTGATCGTAGATCAGTC

		A
		R: AACCGAGAACACCGCGTTAT
L.brevis	16s-23s rRNA	F: ATTTTGTTTGAAAGGTGGCTTCGG
		R: ACCCTTGAACAGTTACTCTCAAAGG
Bacteria	16s	27F: AGAGTTTGATCMTGGCTCAG
		1492R: TACGGYTACCTTGTTACGACTT
	V3-V4 region	341F:TCGTCGGCAGCGTC AGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG
		805R:GTCTCGTGGGCTCGGAGATGTGTA TAAGAGACAG-GACTACHVGGGTATCTAATCC

7. Isolation of *L. sakei* strains from faeces

For the isolation of *L. sakei* strains in faeces, 100 mg of fresh stool was suspended in 900 µL of PBS and homogenised. The homogenised sample solutions were serially diluted (10^{-2}–10^{-6}); 50 µL of each dilution was plated in duplicate on deMan-Rogosa-Sharpe (MRS) medium (BD Difco, MA, U S A) and finally incubated anaerobically at 37 °C for 48–72 h. To exclude non-lactic acid-producing bacteria, each colony with a different morphology was isolated and plated on MRS medium with 1% $CaCO_3$ and incubated anaerobically at 37 °C for 48 hr. Bacteria that produced clear zones around the colonies were selected. The presence of the *L. sakei*-specific *katA* gene was determined via PCR amplification, followed by the isolation of *L. sakei*, using a previously described method [20]. Sanger sequencing of 16S rRNA gene was performed for the confirmation of *L. sakei*. Genomic DNA was isolated from each colony using a

G-Spin Genomic DNA Extraction Kit (iNtRON Biotechnology Inc, Seoul, Korea.), according to the manufacturer's instructions. The isolated genomic DNA was used in a PCR to amplify the 16S rRNA gene using primers 27F-1492R. After purification, using a QIAquick PCR purification kit (Qiagen, CA, U S A), the PCR products were sequenced by Macrogen Inc. (Seoul, Korea). The nucleotide sequences were analysed for sequence similarity using BLAST (http://www.ncbi.nlm.nih.gov/blast), and sequences with a ≥98% match with those in the database were considered to be from the same species the results were further confirmed by using Ribosomal Data Project (RDP) taxonomy classifier. The results showed that only one strain was obtained from the faeces of obese participants.

8. Bacterial Community Analysis

Genomic DNA extraction from 400mg of human stool samples were added to 15 mL of DNA extraction lysis buffer (SDS 4%, Tris-HCL 50mM, EDTA 50mM, NaCl 5000mM) followed by vigorous homogenization by vortexing for 1 minute. 1.4mL of homogenized fecal suspensions were transferred to a 2mL eppendorf tube and followed by bead beating for 50 seconds and centrifuging at 14,000g for 10 minutes. 200μL of supernatants were transferred to 96 well plates and 1μL of supernatants were dissolved in 29 μL of nuclease free water. The plates were kept at -25°C until performing PCR. The extracted DNA was amplified using the primers targeting from V3 to V4 region of bacterial 16s rRNA gene using the primers of 341F and 805R (Table 1). The PCR reaction were performed under the following conditions: initial denaturation at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 sec, primer annealing at 55 °C for 30 sec, and extension at 72 °C for 30 sec, with a final elongation at 72 °C for 5 min. Then, secondary amplification for attaching the Illumina NexTera barcode was performed with i5 forward primer (5'-AATGATACGGCGACCACCGAGATCTACAC-XXXXXXXX-TCGTCGGCAGCGTC-3'; X indicates the barcode region) and i7 reverse primer (5'-CAAGCAGAAGACGGCATACGAGAT-XXXXXXXX-AGTCTCGTGGGCTCGG-

3'). The condition of secondary amplification is equal to the former one except the amplification cycle was set to 8. The PCR product was confirmed by using 2% agarose gel electrophoresis and the amplified products were purified using QIAquick PCR purification kit (Qiagen, CA, U S A) and the equal concentrations of purified products were pooled together and short fragments (non-target products) were removed with Ampure beads kit (Agencourt Bioscience, MA, USA). The product size and the quality were assessed using Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA) using a DNA 7500 chip. Mixed amplicons from different samples were pooled and subjected to pyrosequencing. The process of sequencing was performed by Chunlab, Inc. (Seoul, South Korea) using Illumina Miseq Sequencing system (Illumina, USA) according to the manufacturer's instructions. Pyrosequencing data analysis: Reads obtained from the different samples were sorted by unique barcodes of each PCR product. Then, the sequences of the barcode, linker and primers were removed from the original read of sequences. Among the sequencing reads, reads containing two or more ambiguous nucleotides, reads with low quality scores (average <25) or reads shorter than 300 bp were all discarded. Bellerophone method that compares the BLASTN search results between the forward half and reverse half sequences were used for detecting potential chimeric sequences[27]. After removing the chimeric sequences, each read was assigned to a taxonomic classification in the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net) [28]. This database contains the 16s rRNA gene sequence of type strains with valid published names and representative species level phylotypes from either cultured or uncultured entries in the GeneBank database with complete hierarchical taxonomic classification from the phylum to species. To compare the abundance of taxa among the different groups, the LDA Effect Size (LEfSe; Linear Discriminant Analysis Effect Size) algorithm was used with online interface Galaxy (http://huttenhower.sph.harvard.edu/lefse/). LEfSe analysis was performed using a Kruskal-Wallis sum-rank test with an alpha value of <0.5, followed by the Wilcoxon-rank sum test with an alpha score <0.05 and a one-against-all strategy for multi-class analysis. An effect size >2 (on a log scale) were considered significant in this study.

9. Whole-genome sequencing

For genome sequencing, genomic DNA was extracted from *L. sakei* ob4.1, grown in MRS, using G-Spin Genomic DNA Extraction Kit (iNtRON Biotechnology Inc., Seoul, Korea), according to the manufacturer's instruction. Thereafter, 20-kb sequencing libraries were prepared using PacBio DNA Template Prep Kit 1.0 according to the manufacturer's instructions. The libraries were sequenced using PacBio P6C4 chemistry in 8-well SMRT Cell v3 in PacBio RSII. The sequenced data was assembled with PacBio SMRT analysis 2.3.0 using the HGAP2 protocol (Pacific Biosciences, USA). Contig Construction of library and whole-genome sequencing were performed by ChunLab Inc. (Seoul, Korea). The process of finding genes and functional annotation of whole-genome assemblies was performed using EzBioCloud genome database. Protein coding sequences (CDSs) were predicted by Prodigal 2.6.2[29] and tRNAs were searched using tRNAscan-SE 1.3.1[30]. The search for rRNA and other non-coding RNAs was performed by a covariance model using Rfam 12.0 database[31]. The CDSs were categorised into different groups based on their functions, with reference to orthologous groups (EggNOG 4.5; http://eggnogdb.embl.de)[32]. For comparative genomics, genome sequences of closely related *L. sakei* ob4.1 strain were obtained from the EzBioCloud database[33]. A comparative genomic analysis was conducted using ChunLab's comparative genomics tool (http://www.ezbiocloud.net/contents/cg).

10. Survival rate under the hydrogen peroxide challenge test

Exponential-phase bacterial cells were centrifuged (6,000*g*, 15min), resuspended in MRS medium containing 0, 5, 10, 15, and 30 mM H_2O_2, and incubated at 37 °C. After 1 hr, H_2O_2 was eliminated using bovine liver catalase (10 U/mL, Sigma Aldrich, MO, U S A), and viable cells were counted by plating dilutions on MRS medium.

11. Growth conditions of the *L. sakei* strains

Overnight-grown cultures of *L. sakei* ob4.1 or DSM 20017 were diluted in MRS

medium (1:1000) and incubated at 37 °C either aerobically using a shaking incubator (200 rpm) or anaerobically in an anaerobic jar with GasPak (BD Difco, MA, U S A). CFUs were determined by measuring the optical density at 600 nm every 4 hrs for 3 days.

12. Detection of catalase activity in Lactobacillus strains

Catalase activity was analysed as described previously [34]. Briefly, *Lactobacillus* strains were incubated in MRS medium with 30 μM haematin (Sigma Aldrich, MO, U S A), either aerobically using a shaking incubator (200 rpm) or anaerobically in an anaerobic jar with GasPak (BD Difco, MA, U S A). The exponential growth-phase bacterial cells (OD_{600} = 0.5) were harvested and resuspended in PBS at 10^8 CFU/mL. The resuspended cells were mixed with 0.8 mM H_2O_2. An aliquot was mixed with dichromate in acetic acid, and the samples were boiled and centrifuged to remove the cells. The absorbance was measured at 570 nm. Catalase activity was expressed in terms of μM H_2O_2 degraded per minute per 10^8 CFU.

13. Expression of catalase genes in *L. sakei* strains

RNA isolation and cDNA synthesis were performed as described previously [35]. TRIzol® Max™ Bacterial RNA Isolation Kit (Invitrogen, CA, U S A) was used for RNA isolation, according to the manufacturer's instructions. Sequences encoding the catalase of *L. sakei* ob4.1 were used as templates for primer design (Table 1). The relative expression of the *katA* gene was calculated using the comparative threshold (ΔΔCt) method. *Gapdh* was used as the reference gene.

14. *Caco-2* cell culture

Caco-2 cells (ATCC, MD, U S A) were grown on minimal essential medium (MEM) (Gibco, MA, U S A) containing 10% foetal bovine serum, 1% glutaMAX (Gibco, MA, U S A), 1% MEM non-essential amino acids (Gibco, MA, U S A), and 1% sodium pyruvate at 37 °C and 5% CO_2. After reaching confluency, the cells were seeded into

6- or 96-well plates, for individual experiments, at a density of 1×10^5 cells/well and cultured for 21 days (on average) with medium change every alternate day, until differentiated.

15. Preparation of bacterial suspension

Lactobacillus strains were grown anaerobically in MRS medium for 48 hrs at 37 °C, and bacterial cultures were pelleted down and resuspended in MEM medium (Gibco, MA, U S A) to a final concentration of 10^8 CFU/mL. Heat-killed bacteria were prepared by heating the bacteria at 80 °C for 15 min at a concentration of 1,010 CFU/L in MRS medium. Samples, before and after the killing procedure, were diluted in PBS, plated on MRS agar, and incubated at 37 °C for 72 hrs.

16. Palmitate treatment

Differentiated Caco-2 cells were treated with palmitate (2.5, 5, 7.5 mM; Sigma Aldrich) or fatty acid-free bovine serum albumin (Sigma Aldrich, MO, U S A) and incubated at 37 °C and 5% CO_2 for 6 hrs. Suspensions of *Lactobacillus* strains containing 10^8 CFU/mL cells were added to each well and incubated for another 4 h. The Caco-2 cells were washed twice with DPBS, and cell pellets were resuspended in 1 mL of TRIzol reagent (Molecular Research Centre, OH, U S A) for subsequent RNA extraction

17. Adhesion assay in *Caco-2* cells

Caco-2 cells were sub-cultivated in 6-well plates, as described above, until confluence (3–5 days). The monolayers of cells were washed with DPBS and new cell media, with or without 0.5% H_2O_2, were replaced in each well. Bacterial suspensions were added to each well, with a final concentration of 10^6 CFU in 1 mL of medium and incubated at 37 °C for 1 hr. The cells were washed five times with DPBS to remove non-adherent bacteria. Cells were treated with trypsin-EDTA; the number of viable bacterial cells was determined by spread plate method on MRS, and incubated at 37 °C for 48 hrs. Bacterial adhesion rate was calculated as (Adhered bacteria/Total number of bacteria

added)*100 (%).

18. ROS measurement in *Caco-2* cells

Caco-2 cells were grown in 96-well plate, as described above. The generation of ROS in Caco-2 cells was measured using the Cellular ROS Assay Kit (Abcam, Cambridge, UK), according to the manufacturer's instructions.

19. Mouse experiments

The Institutional Animal Care and Use Committee at the CHA University approved the animal experiments used in this study. Specific pathogen-free male C57BL/6J mice, aged 6 weeks, were fed either 10% control diet (D12450B, Research Diets, Inc., NJ, U S A) or a 60% fat diet (D12492, Research Diets, Inc., NJ, U S A) until the end of the experiment. Eight weeks after starting the diet, 1×10^9 CFU/100 μL of *L. sakei* ob4.1 or DSM 20017 was administered once by oral gavage, and the colonisation of bacteria in mouse faeces was determined by homogenising the latter in 1 mL of sterile PBS, serially diluting the samples, and eventually plating them on MRS medium plates. To investigate the role of a ROS scavenger, mice were given N-acetylcysteine (NAC) (Sigma Aldrich, MO, USA) at 100 mg/L in their drinking water throughout the experiment. To calculate a competitive index for CFUs of *L. sakei* ob4.1 vs. *L. sakei* DSM 20017, two mice were cohoused for 8 weeks, fed high-fat or low-fat diet, and given NAC in their drinking water or regular water. Then, the two mice were separated after an inoculation of 1 × 109 CFU/100 μL of *L. sakei* ob4.1 or *L. sakei* DSM 20017. After the number of bacteria in the mouse colon contents was determined, the competitive index was calculated as the ratio of the number of *L. sakei* ob4.1 over DSM 20017. 4 pairs of mice (8 mice) were used for each experiment. To investigate the long-term administration effect of the strains, four weeks after starting either diet, the mice were supplied with a 1×10^9 CFU/100 μL mixture of the indicated *L. sakei* strains or a mock suspension, which was orally administered every day for 4 weeks. The mice were subsequently euthanised, and samples were collected 24 h after the final infection.

20. Bacterial culture

Spontaneous mutants resistant to rifampin were isolated using the method of Chiaramonte et al[36]. Briefly, *L. sakei* strains were plated onto MRS medium containing 100 mg/L rifampin (Sigma Aldrich, MO, U S A) and incubated at 37 °C for 4–5 days. For supplementation in mice, fresh cultures of *L. sakei ob4.1Rif* and *L. sakei* DSM 20017Rif were grown in MRS broth containing 100 mg/L rifampin. The bacterial cells were pelleted and washed with PBS. The final concentration of each strain was 1×10^9 CFU/mL.

21. Measurement of colon-tissue ROS

The level of ROS in colon tissues was assessed using a Reactive Oxygen Species (ROS) Fluorometric Assay Kit (Elabscience, TX, U S A) using a piece of proximal colon tissue from the mice, according to the manufacturer's instructions.

22. Statistical analyses

For clinical data, data are expressed as the mean ± standard deviation (SD). Categorical data are represented as number (%). A Kolmogorov–Smirnov goodness-of-fit test was performed to determine the population normality of each group. Characteristics between the healthy control group and obesity group were compared using Student's *t* test or chi square test. *Lactobacillus* CFU between the two groups was compared using Mann–Whitney test, and prevalence of positive culture of each strain was compared using Fisher's exact test. The participants were categorised into four groups, based on the inter-quartile ranges of total fat intake, and faecal ROS levels were compared using one-way ANOVA. Odds ratio and 95% CI for the prevalence of positive culture of *L. sakei* species were calculated by multivariate logistic regression analysis after adjustment for age, gender, and BMI. Pearson correlation analysis was performed to evaluate relationship between faecal ROS levels and the relative abundance of *L. sakei* species. For *in-vitro* and mouse experiments, fold changes of

ratios (mRNA levels, faecal ROS levels) and bacterial numbers were transformed logarithmically before analysis to normalise the data. One-way ANOVA, followed by Tukey's multiple comparison tests, were used to determine the differences across the groups. Statistical analyses were performed using the Statistical Package for Social Science version 20.0 (SPSS Inc., IL, USA) and GraphPad Prism 8.0 software (CA, U S A). Statistical significance was defined as $p < 0.05$.

III. RESULTS

1. Clinical characteristics of healthy control and obesity group

In this study, we enrolled 88 adults with obesity and 64 healthy control subjects without obesity. No significant difference was found between the obesity and control groups regarding age, sex, and social habits, except for diet. A higher intake of daily calories, calorie-adjusted fat, and calorie-adjusted saturated fat was found in the obesity group (Table 2).

Table 2. Basic characteristics of the subjects

Characteristics	Control group (n=64)	Obesity group (n=88)	P value
Age (years)	38.28±10.38	40±9.43	0.22
Gender [n (%)]			0.20
Male	31 (48.4)	51 (58.0)	
Female	33 (51.6)	37 (42.0)	
Regular exercise [n (%)]	28 (43.8)	33 (37.5)	0.24
Alcohol Drinking [n (%)]	21 (32.8)	38 (43.2)	0.13
Current smoking [n (%)]	0 (0)	0 (0)	NS
BMI (kg/m^2)	20.20±3.16	28.23±1.14	<0.01
Body fat (%)	24.57±6.11	34.49±4.13	0.02
Total calorie intake (kcal/d	2354.78±118.43	3457.20±315.58	0.01

ay)			
Carbohydrate intake (g/1000kcal/day)	162.23±3.15	205.34±9.78	0.08
Fiber intake (g/1000kcal/day)	14.81±0.67	15.55±0.89	0.23
Protein intake (g/1000kcal/day)	41.21±3.15	46.22±3.57	0.34
Fat intake (g/1000kcal/day)	23.55±3.35	41.25±5.98	<0.01
Saturated fat intake (g/1000kcal/day)	7.34±0.99	19.28±5.01	<0.01
Mono-unsaturated fat intake (g/1000kcal/day)	7.53±1.12	13.57±0.54	0.07
Poly-unsaturated fat intake (g/1000kcal/day)	5.78±1.24	6.38±0.98	0.64

Data are shown as the mean ± standard deviation or the number (percentage).

Abbreviation: BMI, Body mass index; NS, no statistically significant difference.

Energy adjustment was performed by calculating nutrient density and expressed as intake / 1000kcal. P values are calculated by using Student's *t* test or chi square test.

2. *Lactobacillus sakei* was increased in the obesity group

To compare the abundance and composition of *Lactobacillus* species between the two groups, we quantified the relative abundance of *Lactobacillus* in their faeces using real-time PCR and found no difference between the two groups (Figure 1). Considering the high diversity across *Lactobacillus* species, which could obscure the relationship between obesity and *Lactobacillus*, we next performed species-specific PCR analysis. The obesity group was colonised more often with *L. sakei* than the control group (Figure 2). There was no significant difference in the prevalence of other *Lactobacillus* species between the two groups (Figure 2).

Next, we selected the feces samples of 80 participants (47= healthy control group, 33=

obesity group) from among the total 154 participants and further compared the relative abundance of microbiota species between the two groups by analyzing 16S ribosomal RNA gene amplicon sequencing (microbiota profiling). Consistent with previous report[37], the microbiota composition of the obese group was characterized by an increased relative abundance of the phylum Firmicutes and a reduced abundance of the phylum Bacteroidetes compared with healthy subjects. Notably, an elevated relative abundance of the class Clostridia distinguished the fecal microbiota composition of obese subjects from that of the healthy subjects (Figure 3). An elevated abundance of class *Clostridia* is known to be related with high fat diet in obesity[38]. We also compared the relative abundance of fecal microbiota at the species level. Consistent with the results from the species-specific PCR analysis, the microbiota composition from obese subjects was characterized by an increased abundance of the species *L. sakei* compared with the microbiota in healthy subjects (Figure 3).

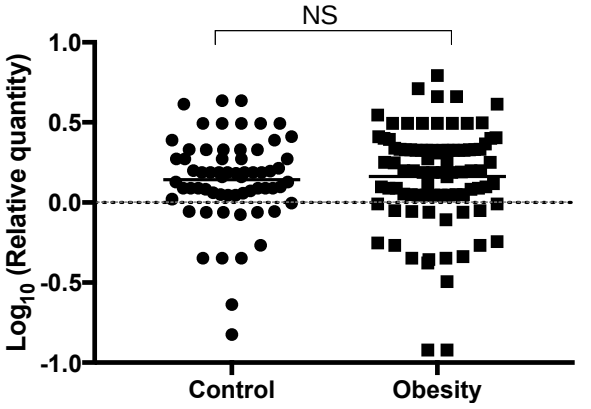

Figure 1. The relative abundance of the genus *Lactobacillus* in faeces of healthy and obese subjects. No difference was found between control and obesity group. NS, not significant.

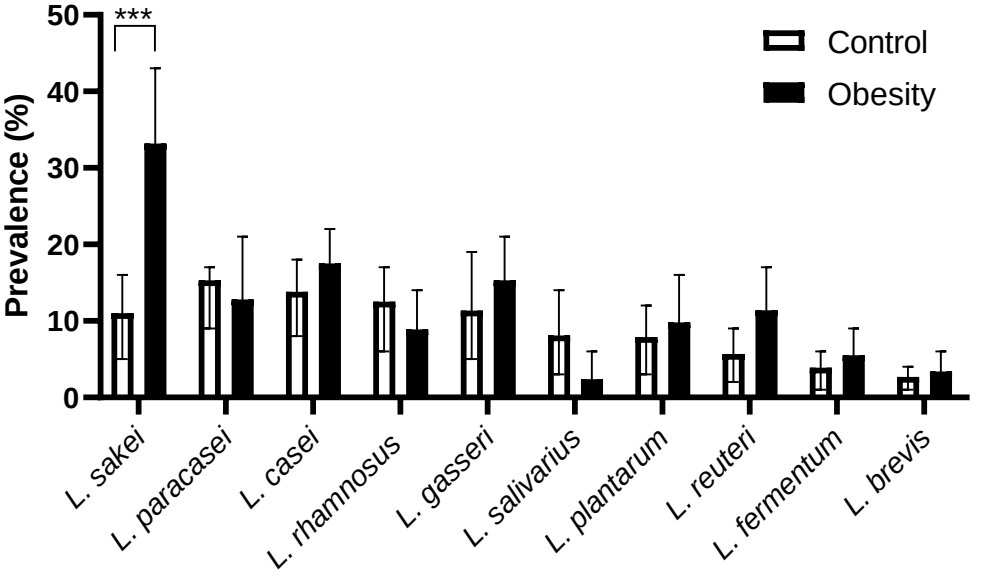

Figure 2. *Lactobacillus* species diversity in healthy and obese subjects. The obesity group was colonised more often with *L. sakei* than the control group. ***, p<0.001.

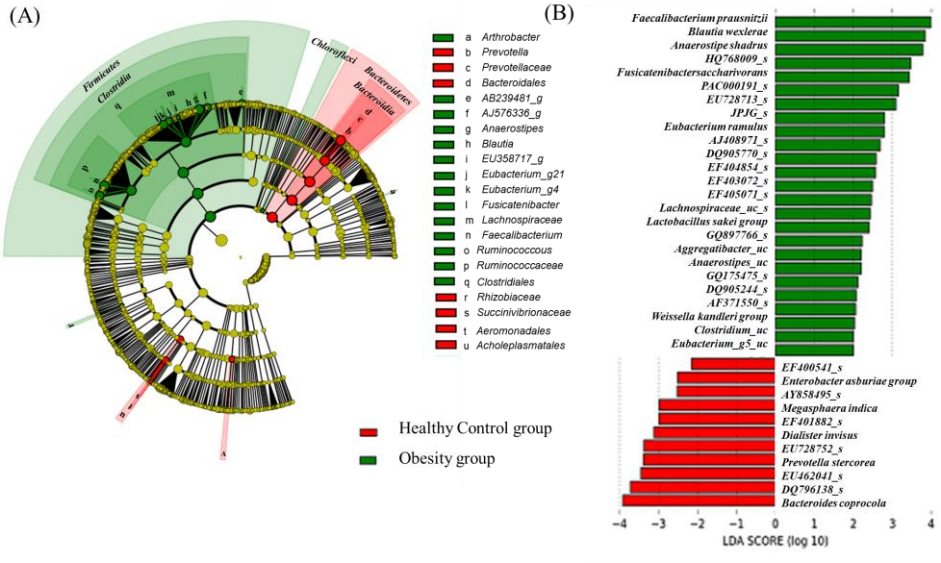

Figure 3. Comparison of the faecal microbiota profile of healthy and obese subjects. Green, elevated in obesity subjects compared to healthy subjects; red,

reduced in obesity subjects compared to healthy subjects. (A) The cladogram shows differences in taxa composition determined by microbiota profiling of faeces of healthy control group and obesity group. (B) Species with significant difference that have an LDA score greater than the estimated value; the default score is 2.0.

3. Relationship between fat consumption, ROS, and *L. sakei* abundance

Since higher fat consumption was seen in the obesity group than in the control group, we analysed the relationship between fat consumption and *L. sakei* abundance. High fat consumption is one of the principal reasons for obesity and affects the composition of the gut microbiota, independent of obesity development [39,40]. We categorised the participants into four groups according to the quartiles of fat consumption (1st quartile, <16.66 g/1000kcal/day ;2nd quartile, 16.66-22.02 g/1000kcal/day; 3rd quartile, 22.02-42.65 g/1000kcal/day; 4th quartile, >42.65 g/1000kcal/day). Notably, participants in the highest fat-consumption group were approximately 3.1 times (odds ratio [OR]: 3.21, 95% confidence interval [CI]: 2.03–4.57) more likely to have the *L. sakei* strain in their faeces than those in the lowest fat-intake group, after adjusting for age, sex, and BMI (Figure 4). Saturated fatty acids increase ROS levels in the gut [6,7] and *L. sakei* contains haem-dependent catalase, despite the fact that most *Lactobacillus* species are catalase negative [19,20]. Hence, we hypothesised that the catalase activity of *L. sakei* is the mechanism that underlies the relationship between a high-fat diet and *L. sakei*. To investigate this hypothesis, we measured ROS levels in the faeces of each participant. We found a trend of increased ROS levels in the faeces from the obesity group compared to that from the control group (p = 0.06) (Figure 5). When categorising the participants according to fat intake, the ROS levels in the faeces were found to increase significantly with the increase in the fat intake, after adjusting for BMI (Figure 6). Therefore, this suggested that the higher levels of ROS might arise from fat intake, rather than from obesity itself. To further investigate the link between faecal ROS and *L. sakei*, we analysed the linear relationship between ROS levels and the relative

abundance of *L. sakei* species in 80 participants. The significant positive relationship ($r=0.293$, $p<0.01$) between the relative abundance of *L. sakei* and ROS levels was found (Figure 7). Although the cross-sectional analysis precluded the determination of a causal relationship, this result suggests that the higher abundance of *L. sakei* found in obese subjects might be associated with the increased colonic ROS levels induced by a high-fat diet.

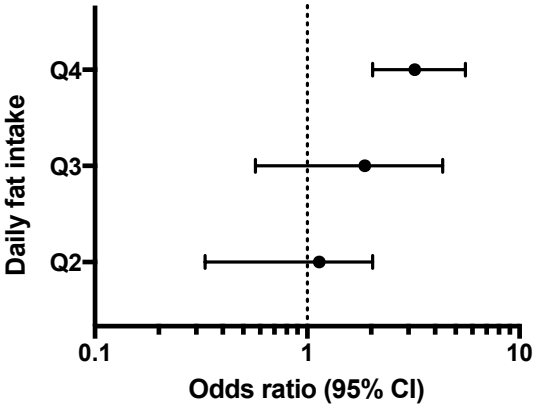

Figure 4. The prevalence of positive culture of *Lactobacillus sakei* according to the amount of daily fat consumption. Participants in the highest fat-consumption group were approximately 3.1 times more likely to have the *L. sakei* strain in their faeces than those in the lowest fat-intake group.

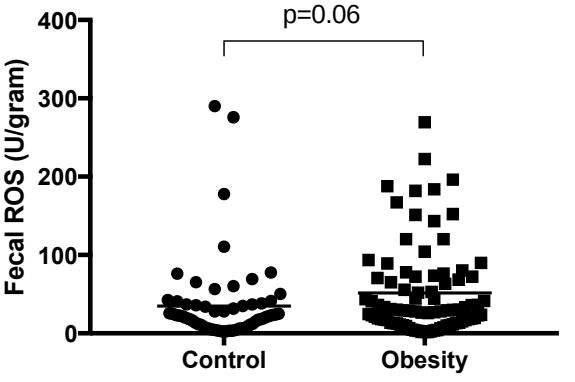

Figure 5. Comparison of the faecal reactive oxygen species levels between healthy

subjects and obese subjects. A trend of increased ROS levels in the faeces from the obesity group compared to that from the control group was found but it was not statistically significant (p=0.06).

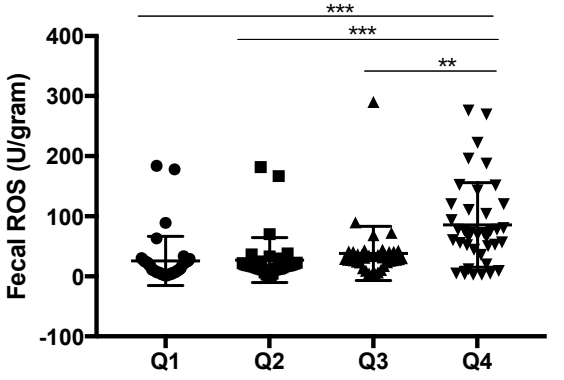

Figure 6. Comparison of the faecal reactive oxygen species according to the amount of daily fat consumption. ROS levels in the faeces increased significantly with the increase in the fat intake, after adjusting for BMI. **, P<0.01; ***, P<0.001.

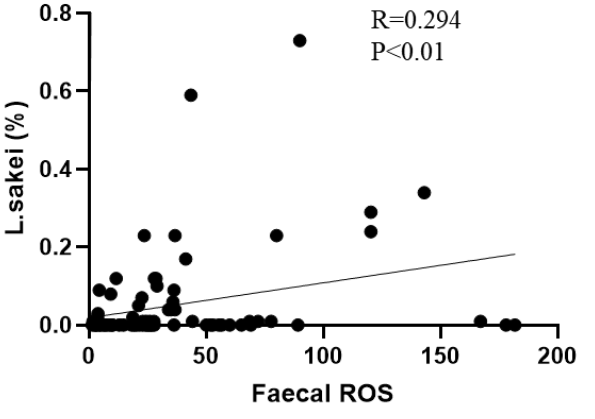

Figure 7. The linear relationship between the abundance of *L. sakei* and faecal reactive oxygen species level. Faecal ROS level was positively correlated with the relative abundance of *L. sakei* (%). P values were calculated by Pearson's

correlation analysis.

4. A *Lactobacillus sakei* strain was isolated from the faeces of obese subjects

To further investigate the characteristics of *L. sakei* related to high-fat diet-induced ROS, we isolated an *L. sakei* strain from the faeces of obese subjects. Fresh faeces from 15 subjects in the obesity group were used for this purpose. *Lactobacillus*-specific culture, for confirming the presence of the *L. sakei*-specific *katA* gene, and 16S rRNA gene sequencing were used for isolation. Only one strain of *L. sakei* was isolated from one subject, and we named it *L. sakei* ob4.1. Severe obesity (BMI 32.15 kg/m^2) and high fat consumption (37.28 g/1000 kcal/day) were found in the host of *L. sakei* ob4.1 (Table 3).

Table 3. Clinical characteristics of the host subject of *L.sakei* ob4.1

Variables	Values
Age (years)	35
Gender	Male
BMI (kg/m^2)	32.15
Regular exercise	Yes
Alcohol drinking	No
Smoking	No
Calorie intake (kcal/day)	3035 kcal/day
Carbohydrate intake (g/1000kcal/day)	194.21
Protein intake (g/1000kcal/day)	43.54
Fiber intake (g/1000kcal/day)	15.67
Fat intake (g/1000kcal/day)	40.28
Saturated fat intake (g/1000kcal/day)	18.46
Mono-unsaturated fat intake (g/1000kcal/day)	11.13
Poly-unsaturated fat intake (g/1000kcal/day)	6.18

Regular exercise was defined as activity more than a moderate degree (walking more than 5

times/week for 30 min, moderate-intensity physical activity more than 5 times/week for 30 min, or vigorous-intensity physical activity more than 3 times/week for 20 min) in recent 1 week. Smoking was defined as being a current smoker and alcohol drinking was defined as drinking alcoholic beverage ≥ 70 g/day, or more frequent than once a week. Energy adjustment was performed by calculating nutrient density and expressed as intake / 1000kcal.

5. *L. sakei* ob4.1 showed higher resistance to oxidative stress than *L. sakei* DSM 20017

Since our data suggested that high fat intake increases ROS levels in the faeces, we aimed to explore the resistance of *L. sakei* ob4.1 against oxidative stress.

L. sakei shows high diversity, regarding catalase activity, across strains [18]. For comparison, we chose *L. sakei* DSM 20017 as a reference strain (isolated from rice wine. For the negative control, we used *L. rhamnosus GG* (ATCC53103), which contained no catalase gene [41]. The survival of each strain was assessed under oxidative stress conditions generated by either H_2O_2 or aeration. The short-term survival ratio of *L. sakei* ob4.1 was significantly higher than that of *L. sakei* DSM 20017 in the presence of each concentration of H_2O_2 (5mM, $p<0.01$; 10mM, $p<0.001$; 15mM, $p<0.001$) (Figure 8, 9). The long-term survival of the *Lactobacillus* strains under aerobic conditions was evaluated next. After 24 h of aerobic growth, the survival of *L. sakei* ob4.1 was approximately 100 times higher than that of *L. sakei* DSM 20017 ($p<0.01$) (Figure 10). Next, we measured the adhesion ability of the *L. sakei* strains, with or without oxidative stress. Compared to *L. rhamnosus GG*, which has a great adhesion ability, both *L. sakei* strains showed similar adhesion properties without H_2O_2. However, after H_2O_2 treatment, *L. sakei* ob4.1 was the only strain that could maintain its adhesion ability and showed significantly higher adhesion property compared with *L. sakei* DSM 20017($p<0.01$) (Figure 11).

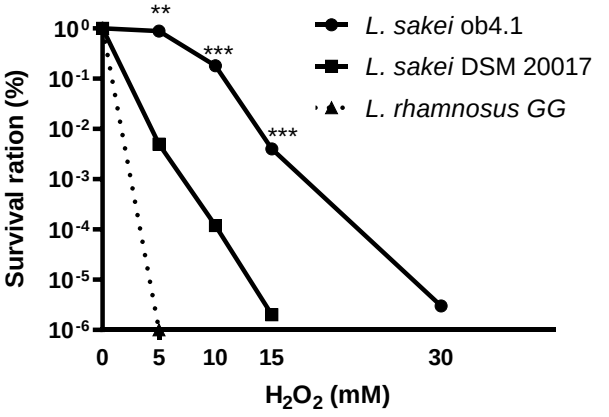

Figure 8. The survival rate of *Lactobacillus* strains under oxidative stress conditions. The short-term survival ratio of *L. sakei* ob4.1 was significantly higher than that of *L. sakei* DSM 20017 in the presence of each concentration of H_2O_2 (5mM, $p<0.01$; 10mM, $p<0.001$; 15mM, $p<0.001$). **, $p<0.01$; ***, $p<0.001$.

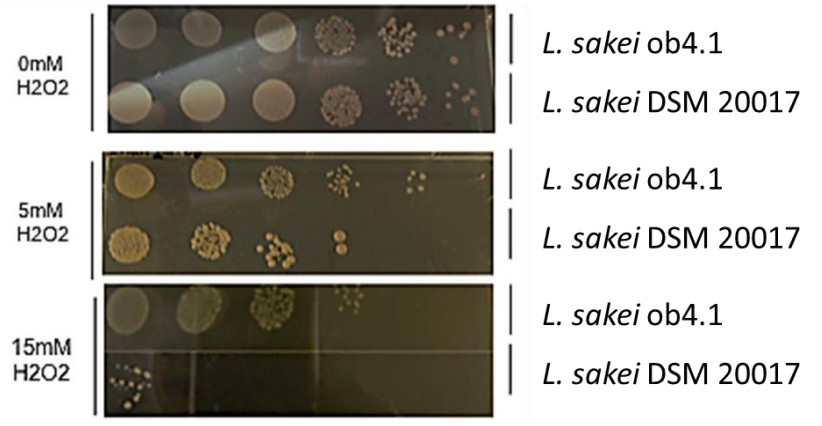

Figure 9. Viable cell numbers of *L. sakei* strains after growth in the presence (5mM, 15mM) or absence of H_2O_2. Serial dilutions of bacterial cells were inoculated onto MRS plates. The growth of the *L. sakei* ob4.1 was significantly higher than that of *L. sakei* DSM20017 in the presence of H_2O_2.

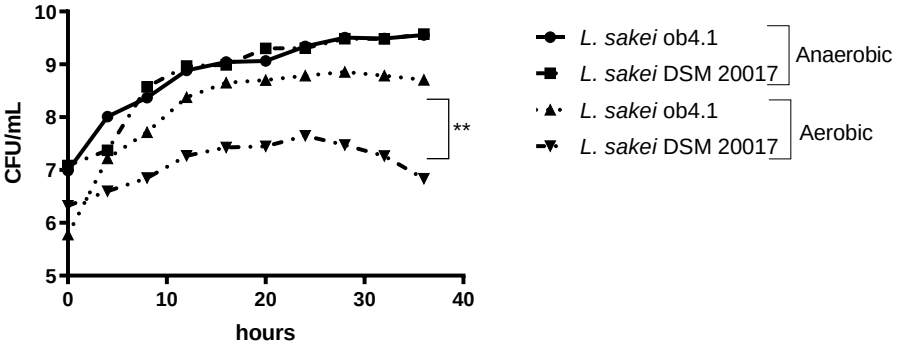

Figure 10. The long-term survival of *L. sakei* strains under aerobic conditions. Overnight-grown cultures of *L. sakei* ob4.1 or DSM 20017 were diluted in MRS medium (1:1000) and incubated at 37 °C, either aerobically using a shaking incubator (200 rpm) or anaerobically in an anaerobic jar with GasPak (BD). CFUs were determined by measuring the optical density at 600 nm every 4 h for 3 days. The long-term survival of *L. sakei* ob4.1 was approximately 100 times higher than that of *L. sakei* DSM 20017. **, p<0.01.

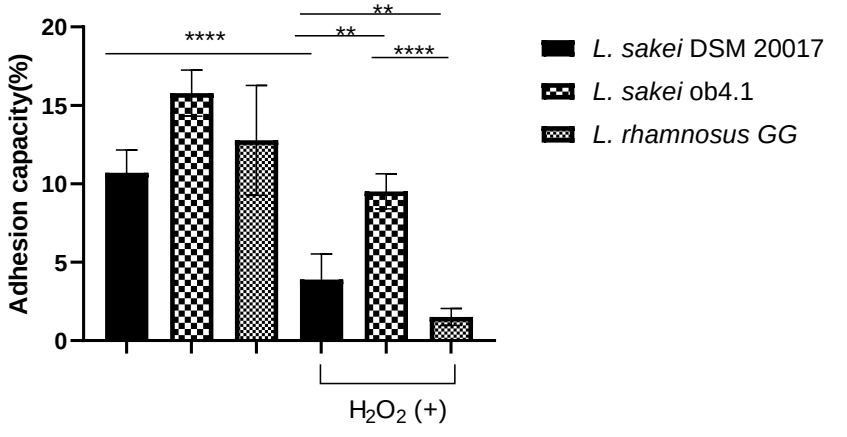

Figure 11. The adhesion ability of *Lactobacillus* strains in the presence or absence of H_2O_2. The adhesion ability was calculated by the percentage of adhered cells with respect to the total number of bacteria. Bars represent the geometric mean ± standard deviation. After H_2O_2 treatment, *L. sakei* ob4.1 showed significantly

higher adhesion ability than those of other strains. **, p<0.01; ****, p<0.0001.

6. Functional and comparative genomic analysis of *L. sakei* ob4.1

To understand the genetic basis of the higher resistance of *L. sakei* ob4.1 to oxidative stress, we sequenced its genome and compared the genes involved in oxidative stress with the genome of *L. sakei* DSM 20017. However, we found no difference in the presence or absence of genes involved in oxidative stress between the two *L. sakei* strains. Each *L. sakei* strain contained one catalase gene (KatA), and 98.5% identity was found across the amino acid sequences of the catalase (Figure 12). 3D structures of catalase proteins were predicted by using trRosetta[42] and two protein structures alignment was performed and compared by using Raptor Structure alignment[43]. 99.37% of structural overlap was found across the alignment of two catalase genes (Root mean square deviation=0.56, Template Modeling score=0.994).

```
98.5% identity in 479 residues overlap; Score: 2522.0; Gap frequency: 0.0%

DSM20017_K      1 MTNQLTTNEGQPWADNQHSQTAGQRGPVLIQDYQLLEKLAHFNRERIPERVVHAKGAGAK
Ob4.1_KatA      1 MTNQLTTNEGQPWADNQHSQTAGQRGPVLIQDYQLLEKLAHFNRERIPERVVHAKGAGAK
                  ************************************************************

DSM20017_K     61 GYFKVTKDMSAYTKAAVFSGVGKKTPLITRFSQVAGEAGYPDTYRDVRGFAVKFYTEEGN
Ob4.1_KatA     61 GYFKVTKDMSAYTKAAVFSGVGKKTPLITRFSQVAGEAGYPDTYRDVRGFAVKFYTEEGN
                  ************************************************************

DSM20017_K    121 YDIVGNNTPVFFVNDPLKFPDFIHSQKRDPRTHARSQDMQWDFWSLSPESVHQVTILMSD
Ob4.1_KatA    121 YDIVGNNTPVFFVNDPLKFPDFIHSQKRDPRTHARSQDMQWDFWSLSPESVHQVTILMSD
                  ************************************************************

DSM20017_K    181 RGIPASYRMMHGFGSHTFKWVNAQGEQFWVKYHFKTNQGIHNLSNELADELAGKDTDYLQ
Ob4.1_KatA    181 RGIPTSYRMMHGFGSHTFKWVNAQGEQFWVKYHFKTNQGVHNLSNELADELAGKDTDYLQ
                  **** ********************************** ********************

DSM20017_K    241 NDLFDAIETGDYPSWTVAVQLVPYEDGLNYPQDIFDVTKVISQKDYPLIEIGQMVLDENP
Ob4.1_KatA    241 NDLFDAIETGDYPSWTVAVQLVPYEDGLNYPQDIFDVTKVISQKDYPLIEIGQMVLDENP
                  ************************************************************

DSM20017_K    301 TNNFEDIEELAFSPANLVPGIEASPDKLLQGRLFGYKDAERYRLGANYEQLPVNRPKVPV
Ob4.1_KatA    301 TNNFEDIEELAFSPANLVPGIEASPDKLLQGRLFGYKDAERYRLGANYEQLPINRPKVPV
                  ************************************************* *******

DSM20017_K    361 HNYERDGAMAQNQATGVNYEPNSQDGPTEVPAAKIHGDQLSGTTGNFSADPDYYSAAGKL
Ob4.1_KatA    361 HNYERDGVMAQNQETGVNYEPNSQDGPTEVPAAKIHSDQLSGTTGNFSTDPDYYSAAGKL
                  ******* ***** ********************** *********** ***********

DSM20017_K    421 YRLLSADEQTRLIENIRMNLGQVTKPEIQIREVKQFYQADPEYGRRVATALNLDLAQFE
Ob4.1_KatA    421 YRLLSADEQTRLIENIRMNLGQVTKPEIQIREVKQFYQADPEYGRRVATALNLDLAQFE
                  ************************************************************
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Figure 12. Amino acid sequence alignment of KatA genes in *L. sakei* ob4.1 and *L.sakei* DSM 20017. *KatA* genes expressed in *L.sakei* ob4.1 and *DSM 20017* were 98.5% identical based on amino-acid sequence.

Figure13. Predictive structure models of catalase enzymes of *L. sakei* ob4.1 (Left) and DSM 20017 (Right) based on the amino sequences.

7. *L. sakei* ob4.1 had higher catalase expression and activity under oxidative stress

We could not identify genetic factors that explained the higher resistance of *L. sakei* ob4.1 to oxidative stress. However, considering that catalase activity is regulated by oxidative stress at gene transcription and protein synthesis levels in various *Lactobacillus* species [19,44], we investigated the effect of oxidative stress on the *katA* mRNA level and catalase enzymatic activity in the *L. sakei* strains. Increased *katA* mRNA expression was detected in both strains, grown in aerobic conditions rather than in anaerobic conditions, and in strains treated with H_2O_2. However, the increase was higher in *L. sakei* ob4.1 (L. sakei ob4.1 vs. DSM 20017 in aerobic condition, $p<0.0001$; under H_2O_2, $p<0.01$) (Figure 13). Next, we measured the catalase activities of the *Lactobacillus* strains. A greater increase in the catalase activity was found in *L. sakei* ob4.1 grown in aerobic conditions (*L. sakei* ob4.1 vs. DSM 20017 in aerobic conditions, $p<0.0001$) (Figure 14). These results collectively suggested that the resistance of *L. sakei* ob4.1 to oxidative stress could be regulated by the extent of oxidative stress at the transcriptional level, rather than at the genetic levels.

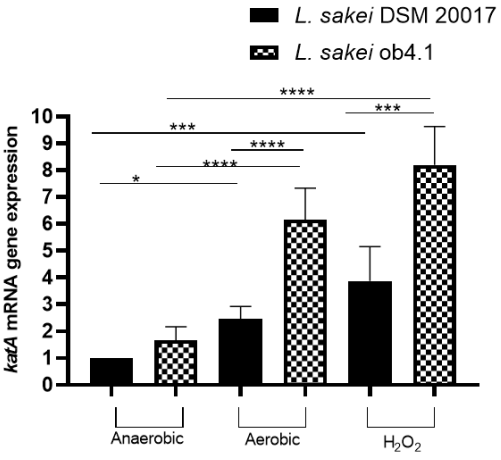

Figure 14. Transcript levels of *katA* genes in *L. sakei* strains with or without oxidative stress. *L. sakei* ob4.1 showed higher increase of catalase *katA* gene expression in response to oxidative stress than *L. sakei* DSM 20017. Bars represent the geometric mean ± standard deviation. *, p <0.05; *; p<0.001; ****, p<0.0001.**

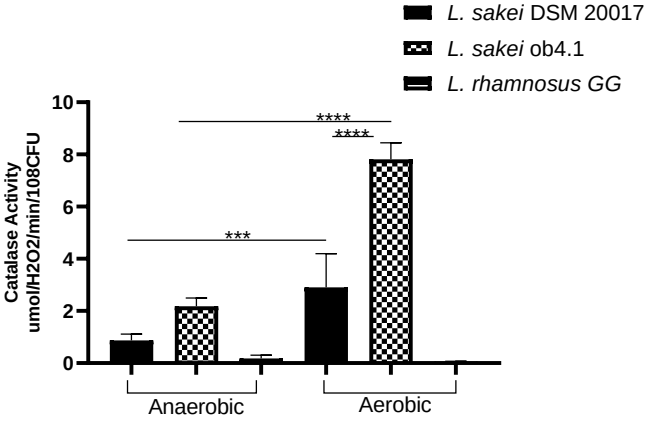

Figure 15. Catalase activities of Lactobacillus strains under aerobic or anaerobic conditions. Catalase activity was expressed as μM H_2O_2 degraded per minute per 10^8 CFU. *L. sakei* ob4.1 showed greater increase of catalase activity in response to aerobic condition compared with other *Lactobacillus* strains. Bars represent the

geometric mean ± standard deviation. ***, p<0.001; ****, p<0.0001.

8. Palmitate increased colon epithelial ROS levels in a dose-dependent manner, and *L. sakei* didn't change the ROS levels

Next, we stimulated Caco-2 cells with a saturated fatty acid (palmitate) to identify the the roles of fat in the host colon epithelium. Palmitate stimulation increased the ROS levels in the colon epithelium in a dose dependent manner (p for trend <0.0001) (Figure 15). However, the high ROS levels were maintained upon treatment with *L. sakei* strain (Figure 16).

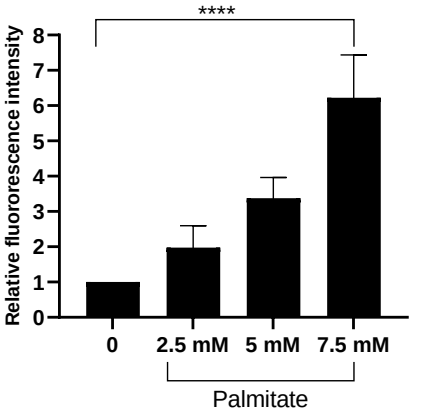

Figure 16. Colon epithelial reactive oxygen species levels under different doses of saturated fatty acid. Palmitate increased colon epithelial ROS levels in a dose dependent manner. ****, p for trend <0.0001. P value is calculated by one-way ANOVA test.

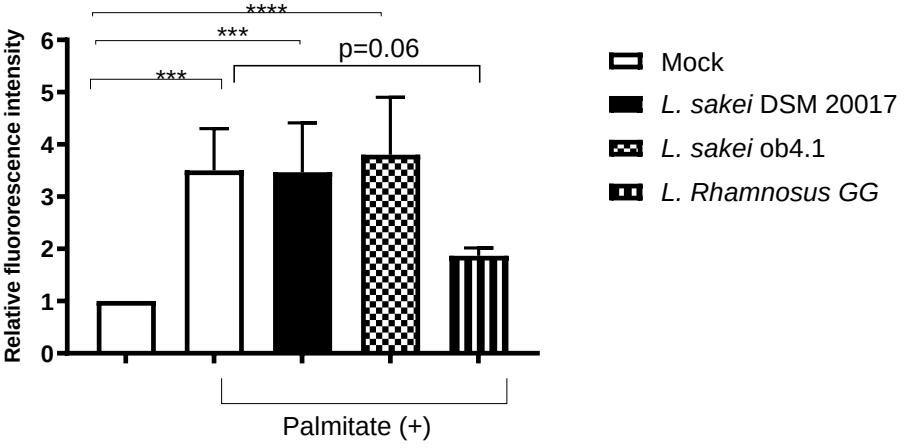

Figure 17. The effects of Lactobacillus strains on saturated fatty acid-induced colon epithelial reactive oxygen species. Palmitate induced colon ROS levels were maintained upon treatment with the *L. sakei* strains ***, p<0.001; ****, p<0.0001.

9. *L. sakei* ob4.1 showed high colonisation and reduced colon inflammation in high-fat diet-induced obese mice

To investigate the interaction between the *L. sakei* ob4.1 strain and host colon *in vivo*, mice maintained on a high-fat (60%) or low-fat (10%) diet for 8 weeks were inoculated independently with 1×10^8 CFU/mouse of spontaneous rifampin-resistant *L. sakei* strains. After 2 days of gavage, the bacterial numbers in the colon contents were counted. An increase in colonisation of *L. sakei* was observed in *L. Sakei* ob4.1-inoculated mice on a high fat diet compared with *L. sakei* DSM 20017-inoculated mice on a high fat diet; bacterial colonisation was increased ~10 fold in *L. sakei* ob4.1-inoculated mice ($p<0.01$). (Figure 17). The total number of *Lactobacillus* was not different between the strains in the mice maintained on a low-fat diet (Figure 17). This result followed the higher attachment ability of *L. sakei* ob4.1 in the H_2O_2-treated Caco-2 cells (Figure 11). Next, to confirm that the high-fat diet–induced ROS caused the higher colonization of *L. sakei* ob4.1 compared with *L. sakei* DSM 20017, we added NAC, an antioxidant that scavenges colon epithelial ROS [45,46] to the drinking water. NAC treatment significantly

reduced high-fat diet–induced colon epithelial ROS levels and eliminated the difference in bacterial colonization between the *L. sakei* ob4.1-inoculated and *L. sakei* DSM 20017-inoculated mice (p=0.48) (Figure 17). Finally, we wanted to determine the causal relationship between obesity and colonisation of *L. sakei*. The mice on the high-fat diet exhibited higher body weight (P<0.001) (Figure 18) and higher colon mucosal ROS levels (p<0.001) (Figure 19) compared with the mice on the low-fat diet. However, following 4 weeks of treatment with *L. sakei* ob4.1 or *L. sakei* DSM 20017, the body weight of the mice and colon mucosal ROS levels did not change, with either a low-fat diet or a high-fat diet (Figure 18, Figure 19).

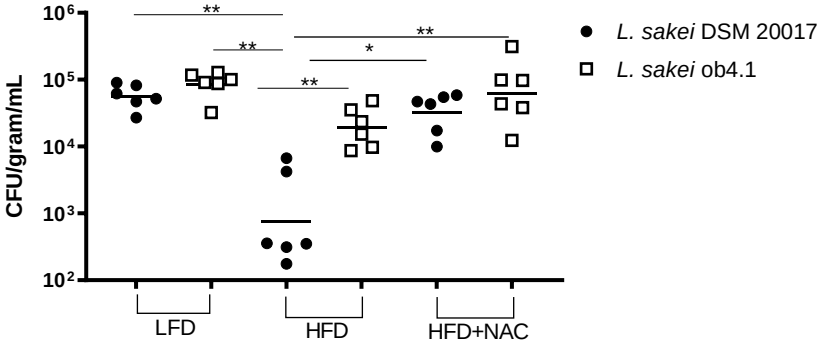

Figure 18. The effects of high-fat diet and reactive oxygen species scavenger on the number of *L. sakei* strain cells colonized in the mouse colon. ~10- fold increase in colonisation of *L. sakei* was observed in *L. Sakei* ob4.1-inoculated mice on a high fat diet compared with *L. sakei* DSM 20017-inoculated mice on a high fat diet. The total number of *Lactobacillus* was not different between the strains in the mice maintained on a low-fat diet or in the mice maintained on a high-fat diet with NAC in drinking water. *, p<0.05; **, p<0.01.

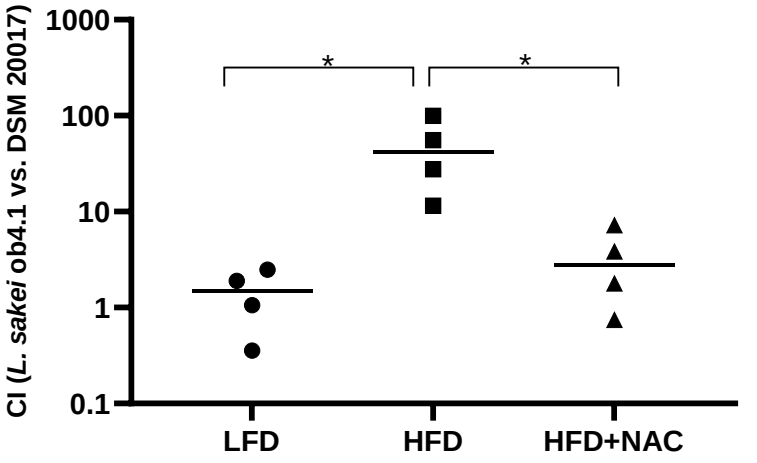

Figure 19. The competitive index for CFUs of *L. sakei* ob4.1 vs. *L. sakei* DSM 20017. The competitive index was calculated as the ratio of the number of *L. sakei* ob4.1 over DSM 20017 and the competitive index was significantly higher in the mice maintained on a high-fat diet compared with the mice maintained on a low-fat diet or the mice maintained on a high-fat diet with NAC in drinking water. *, $p<0.05$

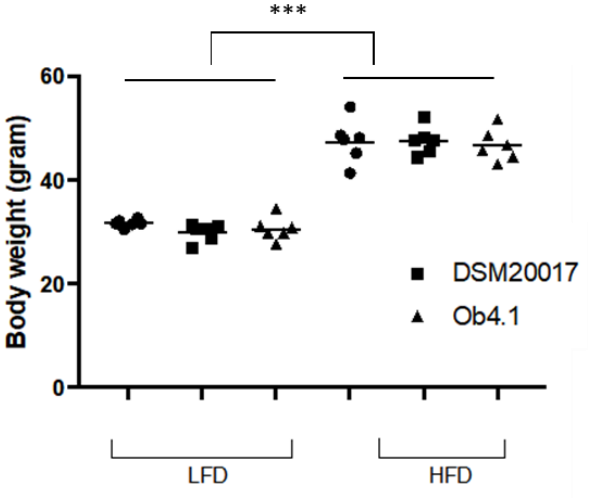

Figure 20. Comparison of body weights between high-fat and low-fat fed mice with or without administration of *L.sakei* strains. 8 weeks of high-fat diet increased

the body weight of the mouse. 4 weeks of treatment with *Lactobacillus strains,* did not change the body weight of the mice with either a low-fat diet or a high-fat diet. ***, p<0.001

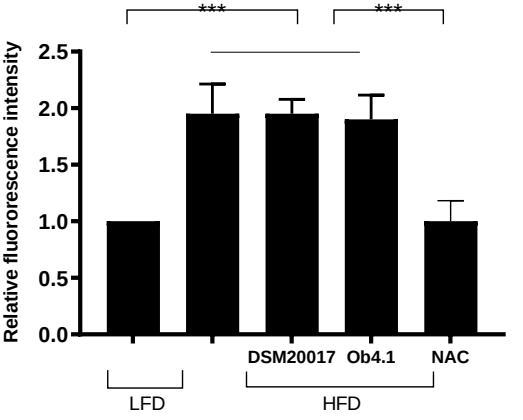

Figure 21. The effects of high-fat diet and the administration of *L. sakei* strains on the colon reactive species levels . NAC treatment significantly reduced high-fat diet induced colon epithelial ROS levels but 4 weeks of treatment with *L. sakei* ob4.1 or *L. sakei* DSM 20017 did not change the colon mucosal ROS levels with either a low-fat diet or a high-fat diet Bars represent the geometric mean ± standard deviation. ***, p<0.01.

IV. DISCUSSION

L. sakei belongs to the genus *Lactobacillus*, which is present in raw meat and widely used as a starter for the fermentation of sausage [47,48,49]. Recently, *L. sakei* has been found in human faeces [50,51] and could be related to the human diet, such as the consumption of meat. In addition, a higher abundance of *L. sakei* has been reported in the obese population compared with the non-obese population, although the underlying mechanism for the difference remains unknown. In this study, we found a higher abundance of *L. sakei* in the obese group using two different methods: species-specific PCR and 16S ribosomal RNA gene amplicon sequencing. Our results are consistent

with previous findings. Furthermore, we found high-fat diet–induced ROS to be a potential factor linking obesity and gut microbiota. A high-fat diet increases the levels of colon epithelial ROS [6,7] and induces colon microbial dysbiosis [8,9]. We also found a significant relationship between the relative abundance of *L. sakei* and fecal ROS levels. Our results provide experimental support for the idea that high-fat diet–induced colon ROS is responsible for an increased abundance of *L. sakei* in obesity.

Although most *Lactobacillus* strains are sensitive to oxidative stress caused by H_2O_2, *L. sakei* is a strain that is highly resistant to the same via catalase activity [19,20]; there is, however, a high degree of intraspecies diversity regarding the response to oxidative stress [18]. In our study, we isolated an *L. sakei* strain *(L. sakei* ob4.1*)* from the faeces of humans with severe obesity; the strain showed higher resistance to oxidative stress relative to that shown by a reference strain *L. sakei* DSM 20017 isolated from rice wine. Oxidative stress occurs because of an imbalance between reactive oxygen species and the defence system responsible for ROS elimination. A long-term high-fat diet induces oxidative stress in the colon [6,7] besides inducing bacterial dysbiosis by changing the redox status [8,9]. Bacteria with higher resistance to oxidative stress (e.g. *E. coli*) have a higher chance of survival [9]. In our study, *L. sakei* ob4.1*,* with higher resistance to oxidative stress, showed a greater survival rate than *L. sakei* DSM 20017 *in vitro* as well as higher colonisation in the colon of high-fat diet-fed mice. These results collectively suggested that the higher catalase activity of *L. sakei* ob4.1 enables the strain to survive better in the colon under high oxidative stress. We could not find the precise molecular basis that could explain the higher catalase activity of *L. sakei* ob4.1; however, the increased gene transcriptional levels and catalase activity under oxidative stress suggested the possibility of it being regulated, at transcriptional and translational levels, by the level of oxidative stress.

In our study, 4 weeks of treatment with *L. sakei* ob4.1 did not increase the body weight or colon ROS levels of mice fed either a low-fat diet or a high-fat diet. The risk that ingesting Lactobacillus strains as probiotics could promote obesity has been suggested [52]. However, insufficient evidence supports that relationship, and the suggestion

remains controversial [15,53]. Although we cannot generalize the results from a single strain of Lactobacillus isolated from one obese person, our results do not support the hypothesis that Lactobacillus strains can cause obesity.

The current study had certain limitations. We did not find the causality for the cross-sectional relationship between the *Lactobacillus* strains and the obesity group. We compared *L. sakei* ob4.1 with *L. sakei* DSM 20017, a reference strain isolated from food, rather than with a strain isolated from the control group. Additionally, we isolated a single *L. sakei* strain from one obese participant, which does not allow for generalization of our data to the entire obese population.

V. CONCLUSION

A higher abundance of *L. sakei* correlated with high-fat diet–induced fecal ROS in an obese population. The higher catalase activity of the *L. sakei* ob4.1 strain, which we isolated from an obese subject, enables the strain to survive better than other strains in the colon under high oxidative stress. Thus, a high-fat diet induces changes in the colon environment by increasing ROS levels, thereby providing a colonization benefit for *L. sakei* strains with higher catalase activity.

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ABSTRACT(IN KOREAN)

고지방 식이로 유도된 산화 스트레스가 비만에서 락토바실러스 사케이균의 장 내 생착율에 미치는 영향

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이지연

비만은 다양한 만성 질환의 위험 인자로 전세계적으로 발생률이 계속 증가하고 있는 중요한 공공 건강 문제이다. 비만과 장 내 공생 미생물 간의 관련성에 대한 연구는 최근 들어 활발히 이루어지고 있으며, 이 중 비만한 집단의 장에서 일부 락토바실러스 균 종의 검출률이 증가하였다는 연구가 수차례 발표된 바 있어, 락토바실러스 균이 비만에 어떠한 영향을 미치는지에 대한 관심이 지속되고 있다. 하지만 아직 락토바실러스 균과 비만과의 관련성의 인과 관계나 기전은 잘 알려지지 않았다. 본 연구에서는 64명의 비만하지 않은 건강한 대조군과 80명의 비만군의 대변을 분석하여, 비만 관련 락토바실러스 균 종을 조사하였다. 그 결과 락토바실러스 사케이균이 비만군의 대변에서 더 많이 검출되었으며, 이는 비만과 관련된 여러 인자 중 고지방 식이로 유도된 산화 스트레스 발생량과 유의미한 상관 관계를 갖고 있었다. 우리는 비만 집단에서 수집한 대변에서 한 종류의 락토바실러스 사케이 균을 분리 동정하여, 이를 락토바실러스 사케이 ob4.1 로 명명하고, 이 균의 유전적, 분자생물학적 특성을 표준 대조 균주로 이용되는 락토바실러스 사케이 DSM 20017 균주와 비교하였다. 그 결과 락토바실러스 사케이 ob4.1 균은 상대적으로 산화 스트레스에 대한 높은 저항 능력을 가지고 있었으며, 이 능력은 산화 스트레스 자극에 반응하여 유전자 전사 단계에서 조절되는 것을 확인하였다. 또한 산화 스트레스 자극이 존재하는 환경에서 표준 균주와 비교하여 더 높은 장 상피

부착률을 보여, 산화 스트레스가 증가된 장 내 환경에서 장 내 생착률이 높을 가능성을 보여주었다. 포화 지방산을 이용하여 장 상피 세포를 자극한 결과, 포화 지방산이 투여된 농도에 비례하여 장 상피의 산화 스트레스가 증가함을 확인하였으나, 락토바실러스 사케이 ob4.1 균의 투여가 산화 스트레스 양의 변화에는 관여하지 않았다. 마지막으로, 고지방을 투여하여 비만을 유도한 마우스에, 락토바실러스 사케이 ob4.1균 및 DSM 20017 균을 투여한 후 장 내 검출량을 비교한 결과, 락토바실러스 ob4.1균의 상재율이 DSM 20017균에 비해 증가함을 확인하였다. 고지방 식이는 마우스의 체중 및 장 내 산화 스트레스양을 유의미하게 증가시켰으나, 4주간의 락토바실러스 사케이 ob4.1균의 투여는 마우스의 체중 변화나 산화 스트레스 량의 변화에는 영향을 주지 않았다. 이러한 결과는 고지방 식이로 인해 유도된 산화 스트레스 증가라는 장 내 환경의 변화가 상대적으로 산화 스트레스에 대한 저항력이 높은 락토바실러스 사케이 균주의 장 내 생존 및 생착 확률을 높여, 락토바실러스 사케이 균이 비만에서 더 높은 검출률을 보여주었을 가능성을 시사해주고 있다.

핵심되는 말: 락토바실러스 사케이, 비만, 장 내 공생 미생물, 산화 스트레스, 고지방 식이

PUBLICATION LIST

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