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Full Paper

Lactococcus kimchii extends lifespan and alleviates motility decline in *Caenorhabditis elegans* through *ins-20*, an insulin-like peptide gene

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***Lactococcus kimchii* is isolated from commercial kimchi, which is a traditional Korean fermented food. This study was conducted to evaluate the probiotic effects of *L. kimchii*. *Caenorhabditis elegans* was fed *L. kimchii*, and its longevity, motility, and gene expression were examined. When fed a 1:1 mixture of *Escherichia coli* OP50 and *L. kimchii* (OP+LK), *C. elegans* had a significantly longer lifespan and increased locomotion than when it was fed OP alone. There was no significant difference in brood size between the OP+LK and OP groups, suggesting that these effects occurred in a dietary restriction-independent manner. RNA sequencing and Gene Ontology analysis showed that the expression of *ins-20*, an insulin-like peptide and agonist of the insulin receptor, was significantly upregulated in the OP+LK group. The *ins-20* mutation annulled the effects of OP+LK on lifespan extension and motility. In addition, OP+LK failed to extend the lifespan of *C. elegans* deficient in *daf-2*, a receptor for the insulin-like signaling pathway. These results suggest that *L. kimchii* extends the lifespan and alleviates motility decline in *C. elegans* through the insulin signaling pathway, highlighting the potential of using *L. kimchii* as a beneficial bacterium for probiotics and postbiotics.**

Key words: *Lactococcus kimchii*, *Caenorhabditis elegans*, longevity, motility, insulin-like signaling pathway

INTRODUCTION

A probiotic is a live microorganism that provides health benefits to a host when administered in appropriate doses. Probiotics function by modifying the gut microbiota, competitively adhering to the mucosa and epithelium, enhancing the gut epithelial barrier, and modulating the immune system [1]. A postbiotic is defined as a "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host", and postbiotics have also attracted attention in recent years [2]. The genera most frequently used as probiotics or postbiotics include *Lactobacillus* and *Bifidobacterium* [3, 4].

Kimchi, a traditional Korean fermented food, is prepared by fermenting Baechu cabbage with other vegetables; lactic acid bacteria predominate during the fermentation process. Kimchi consumption has been reported to have various health benefits [5]. Administration of kimchi to high-fat diet-induced obese mice exhibited anti-obesity effects by reducing body weight gain and adipose tissue weight [6]. In a study of humans, systolic and diastolic blood pressure, percent body fat, fasting glucose, and total cholesterol were significantly improved in a fermented kimchi intake group compared with a raw kimchi intake group [7].

Lactococcus kimchii has been isolated from commercial kimchi [8]. *Lactococcus* spp. exhibit probiotic and postbiotic effects and may be beneficial to living organisms. Oral administration of *Lactococcus lactis* ML2018 in a mouse model of colitis significantly improved colitis by reducing body weight and preventing epithelial cell apoptosis [9]. Moreover, feeding *L. lactis* strains to carp effectively improved their growth and activated innate immunity [10]. In addition, supplementing broiler diets with *Lactococcus garvieae* B301 increased body weight and

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the abundances of cecum lactic acid bacteria and *Bifidobacterium* spp. and decreased the feed-to-gain ratio and abundance of cecum coliforms [11]. Heat-inactivated *Lactobacillus gasseri* strain CP2305 improved sleep quality [12] and reduced anxiety and sleep disturbance [13].

Caenorhabditis elegans is a useful experimental animal model, as it feeds on bacteria and has a short life cycle (3.5 days at 20°C) and adult hermaphrodites produce a large number of offspring through self-fertilization [14]. Therefore, *C. elegans* is a useful model for studying lifespan and aging. Although specific intestinal microbes of wild *C. elegans* have been described [15, 16], *C. elegans* is typically maintained on agar plates in the laboratory and fed laboratory *Escherichia coli* strains (e.g., OP50) [17]. Lactic acid bacteria prolong the lifespan of *C. elegans*, whereas pathogenic bacteria, such as *Salmonella enterica* and *Staphylococcus aureus*, shorten the lifespan of *C. elegans* [18]. *C. elegans* is a promising model organism for examining the effects of a target bacterium on its host. Feeding of *Lactococcus cremoris* subsp. *cremoris* to *C. elegans* extends their lifespan and alleviates reduced motility [19]. Furthermore, both live and heat-killed *L. cremoris* prolonged the lifespan of *C. elegans* compared with that of heat-killed OP50-fed nematodes [19]. Therefore, *L. kimchii*, shows potential as a probiotic and postbiotic. However, its beneficial effects on health have not been reported. This study was conducted to examine the effects of *L. kimchii* on the longevity and motility of *C. elegans*.

MATERIALS AND METHODS

Bacterial strains and culture conditions

The standard diet for cultivating *C. elegans* consisted of *E. coli* strain OP50 (OP), which was obtained from the Caenorhabditis Genetics Center and cultivated on tryptone soya growth media (Nissui Pharmaceutical, Tokyo, Japan) at 37°C for 24 hr. The *L. kimchii* (NBRC113348) strain was obtained from the National Institute of Technology and Evaluation (Tokyo, Japan) and cultured anaerobically on de Man, Rogosa, and Sharpe growth media (Kanto Chemical, Tokyo, Japan) at 30°C for 48 hr. To weigh cultures, the bacteria grown on the media were gently scraped using a sterile inoculating loop and transferred to a 1.5 mL tube. Suspensions of the collected bacterial cultures (20 mg wet weight) was prepared in 0.5 mL of M9 buffer (5 mM potassium phosphate, 1 mM $CaCl_2$, and 1 mM $MgSO_4$). For experiments, 50 µL of bacterial suspension was disseminated on peptone-free modified nematode growth media (mNGM) consisting of 1.7% w/v agar, 50 mM NaCl, 1 mM $CaCl_2$, 5 µg/mL cholesterol, 25 mM KH_2PO_4, and 1 mM $MgSO_4$. Each 5-cm mNGM plate contained 2 mg of bacterial feed culture.

Caenorhabditis elegans strains and culture conditions

The Caenorhabditis Genetics Center, University of Minnesota, supplied the *C. elegans* Bristol strain N2 (wild type) and its derivative mutant strain CB1370 *daf-2* (*e1370*). FX01947 *ins-20* (*tm1947*) and FX05634 *ins-20* (*tm5634*) were obtained from the National Bioresource Project for *C. elegans*, Japan. Nematodes were cultured and propagated on a nematode growth medium (NGM) as previously described [17]. The *daf-2* mutants were maintained at 20°C or lower to prevent dauer entry. To obtain eggs, mature nematodes were treated with a mixture of sodium hypochlorite and sodium hydroxide. The egg suspension was incubated in M9 buffer at 25°C (20°C for *daf-2* mutants) for 1 day to promote hatching and synchronization. The synchronized L1-stage worm suspension was centrifuged at 156 × *g* for 1 min. After removing the supernatant via aspiration, the remaining larvae were used for subsequent assays.

Determination of C. elegans *lifespan*

For lifespan analysis, L1-stage worms were cultured on mNGM plates covered with 10 mg of OP at 25°C for 2 days (20°C, 4 days for *daf-2* mutants) until they reached the young adult stage (referred to as 3-day-old nematodes). Synchronized 3-day-old nematodes (35 animals per plate) were placed on 5-cm mNGM plates covered with 2 mg of OP alone or a mixture (1:1) of OP and *L. kimchii* (LK) and incubated at 25°C (20°C for *daf-2* mutants). Worms were transferred daily to fresh plates for the first four days and every other day thereafter. The numbers of live and dead worms were recorded daily. A worm was considered dead if it failed to respond to a gentle touch with a worm picker. Worms that crawled off the plate or died from internal hatching were considered lost and were excluded from the analysis. The assay for N2 animals (Fig. 1A) was performed in duplicate, and the replicated statistics were merged. Worm survival was calculated using the Kaplan–Meier method.

Mean lifespan was estimated using the following formula [20]:

$$Mean\ lifespan = \frac{1}{N}\sum_j \frac{X_j + X_{j+1}}{2} dj,$$

where d_j is the number of worms that died in the age interval (x_j to x_{j+1}) and N is the total number of worms. The standard error (SE) of the estimated mean lifespan was calculated using the following equation:

$$SE = \sqrt{\frac{1}{N(N-1)}\sum_j \left(\frac{X_j + X_{j+1}}{2} - MLS\right)^2 d_j}.$$

Maximum lifespan was calculated as the mean lifespan of the longest-living worms (15%) in each group.

Locomotory score of C. elegans

Young adult worms (3 days old) were plated on mNGM plates containing OP lawns or a mixture of OP and LK. The dishes were incubated at 25°C (20°C for *daf-2* mutants). Worm motility was measured at various ages using a previously described scoring technique [21, 22]. Briefly, nematodes were categorized as "class A" if they moved vigorously or spontaneously in reaction to being prodded, "class B" if they appeared to move incoherently or did not move unless prodded, and "class C" if they only moved their heads and/or tails in reaction to being prodded. Nematodes that died were categorized as "class D". The combined data were statistically examined.

Measurement of brood size

One L4-stage hermaphrodite was transferred to an mNGM plate covered with a lawn of OP alone or a mixture (1:1) of OP and LK. Parental nematodes were transferred to fresh mNGM plates every 24 hr until the reproductive period was terminated. The resulting eggs were allowed to hatch, and the number of progeny was counted.

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Measurement of body size

Three-day-old adult worms were placed on mNGM plates covered with a lawn of OP alone or with a mixture (1:1) of OP and LK. The plates were incubated at 25°C. The body sizes of live worms were measured every 48 hr until the worms reached 11 days of age. Images of mature nematodes were captured and analyzed using a BZ-X800 microscope (Keyence, Tokyo, Japan) and the Hybrid Cell Count software (BZ-H3C, Keyence). The area of the worm projection was used as an index of body size.

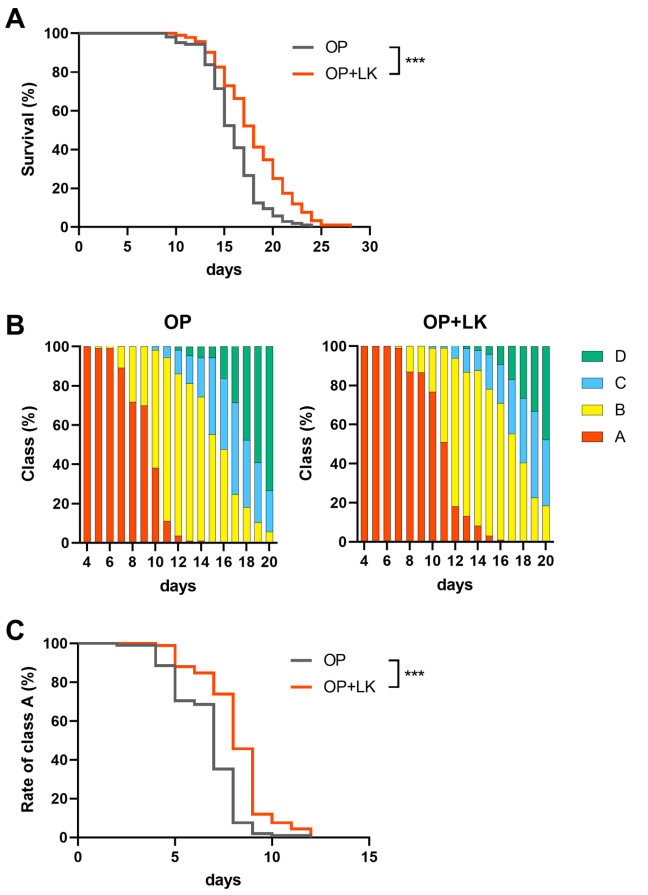

Fig. 1. *Lactococcus kimchii* (LK) extended the lifespan and increased the motility of *Caenorhabditis elegans*. (A) Survival curves of *C. elegans* fed *Escherichia coli* OP50 alone (OP, n=105) or a mixture (1:1) of OP50 and LK (OP+LK, n=92). Day 0 of observation was set as the day on which young adults were 3 days old. Survival rates were calculated using the Kaplan–Meier method, and survival differences were compared using the log-rank test. ***p<0.001. Detailed lifespan data and statistics are provided in Table S6. (B) Classification of locomotory activity of LK-fed *C. elegans*. Worms of each age were classified into the following four classes based on their locomotion: class A, robust and coordinated sinusoidal locomotion (red bars); class B, uncoordinated and/or sluggish movement (yellow bars); class C, no forward or backward movement but head movements or shuddering in response to prodding (blue bars); and class D, dead animals (green bars). The figure shows the percentage of worms in each class at the indicated time points. OP, n=105; OP+LK, n=92. (C) Healthspan curves of *C. elegans* in the OP and OP+LK groups. The proportion of class A worms over time was plotted. Class A proportions were calculated using the Kaplan–Meier method, and differences in proportions between the class A and control groups were compared using the log-rank test. ***p<0.001. Detailed healthspan data and statistics are provided in Supplementary Table 7.

Detection of intestinal **L. kimchii** *in* **C. elegans**

The presence of *L. kimchii* in the intestinal tract of *C. elegans* was evaluated as described previously [23], with minor modifications. Approximately 100 µL of *L. kimchii* cell pellets cultured in de Man, Rogosa, and Sharpe broth media were incubated for 1 hr in 1 mL of phosphate-buffered saline containing 0.1 mg of fluorescein isothiocyanate (FITC; Dojindo, Kumamoto, Japan). The cells were washed 3 times with phosphate-buffered saline, resuspended in M9 buffer, and spread onto an NGM plate. After young adult-stage worms were placed on an FITC-labeled *L. kimchii* NGM plate for 2.5 hr, fluorescence was captured using a microscope (BX53, Olympus, Tokyo, Japan).

RNA extraction and sequencing

Three-day-old worms were cultured for one day on mNGM plates covered with a lawn of OP alone or a mixture (1:1) of OP and LK. Approximately 200 worms per group were collected, washed at least three times with M9 buffer containing 0.2% gelatin, and soaked in RNAlater solution (Qiagen, Hilden, Germany). These samples were stored at −80°C until RNA extraction. Thawed nematode suspensions were ground using a microtube pestle (Scientific Specialties, Lodi, CA, USA) and homogenized in TRIzol (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA was isolated using an RNeasy Mini Kit (Qiagen). RNA sequencing was performed as previously described [24], with some modifications. After obtaining the read count of gene features using the featureCounts tool (version 2.0.1) [25], a quantitative differential expression analysis between conditions was performed using DESeq2 (version 1.20.0) [26] to compare the control and OP+LK-fed groups. Genes with a $\log_2$ fold-change of ≥2 and those with a $\log_2$ fold-change of ≤−2 and a base mean of ≥5 (Supplementary Tables 1 and 2) were subjected to an enrichment analysis based on their Gene Ontology (GO) biological process, molecular function, and cellular component categories using clusterProfiler (version 3.16.0; Supplementary Tables 3 and 4).

Reverse transcription and real-time polymerase chain reaction

After removing genomic DNA, cDNA was synthesized from total RNA using a QuantiTect Reverse Transcription Kit (Qiagen). Real-time quantitative polymerase chain reaction (PCR) was performed in a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific) using Power SYBR Green PCR Master Mix (Thermo Fisher Scientific). The reaction parameters were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min. Samples from three biological replicates were analyzed. Relative mRNA expression was determined using the ΔΔCt method [27] and normalized to the expression of three housekeeping genes (*act-1*, *tba-1*, and *cyc-1*). The primers used for real-time PCR are listed in Table S5.

Statistical analyses

Statistical analyses were performed using the GraphPad Prism 8 software (Ver. 8.4.3, GraphPad, San Diego, CA, USA). The unpaired two-tailed t-test was used to compare gene expression and measurements of brood and body size. The log-rank (Mantel–Cox) test was used to compare survival curves and motility ratings.

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RESULTS

Effect of L. kimchii *on the lifespan and locomotion of wild-type (N2)* C. elegans

We first investigated whether feeding *L. kimchii* affected the longevity of *C. elegans*. Because the nematodes avoided the *L. kimchii* strain, a 1:1 mixture of *E. coli* OP50 and *L. kimchii* (OP+LK) was fed to the nematodes. Wild-type nematodes fed OP+LK showed a significantly ($p<0.001$) longer lifespan than those fed *E. coli* OP50 (OP, Fig. 1A).

The rate of locomotion is closely associated with lifespan. To determine whether *L. kimchii* alleviates the age-related decline in locomotion, we evaluated the locomotory abilities of the nematodes. Similar to the effect on survival, the OP+LK diet did not inhibit the locomotion of *C. elegans*. The locomotor scores of nematodes fed OP+LK were significantly higher than those of nematodes fed OP (Fig. 1B, 1C). Independent duplicate experiments both revealed a significantly prolonged lifespan and increased motility (Supplementary Fig. 1).

Evaluating ingestion of L. kimchii *by* C. elegans

Dietary restriction presumably increases the longevity of different animal species [28], including *C. elegans* [29]. Thus, we examined whether the nematodes ingested *L. kimchii*. To detect intestinal *L. kimchii* in the nematodes, FITC-labeled *L. kimchii* was fed to them. The results showed that FITC was detected in nematodes fed OP+LK (Fig. 2A). The body sizes of the nematodes fed OP+LK were measured at different experimental stages and compared with those of the OP-fed groups. The results showed that body size was significantly larger in the OP-fed group than in the OP+LK group ($p<0.01$) at 5 and 7 days of age; no significant difference was observed after 9 days of age (Fig. 2B). The brood size of nematodes fed OP+LK did not significantly differ from that of OP-fed nematodes (Fig. 2C).

Analysis of genes upregulated by L. kimchii *feeding*

RNA sequencing was performed to identify the genes regulated by *L. kimchii* feeding. GO analysis was performed to detect genes that were upregulated in the OP+LK group, and mRNA expression levels were evaluated using real-time PCR. GO analysis of genes with more than a four-fold increase in expression following feeding of *L. kimchii* revealed six significantly enriched genes with GO terms in the biological process category: chondroitin sulfate proteoglycan biosynthetic process, chondroitin sulfate proteoglycan metabolic process, proteoglycan biosynthetic process, glycoprotein biosynthetic process, proteoglycan metabolic process, and regulation of signaling receptor activity (Supplementary Table 3). The specific genes involved were *glct-3*, *T15D6.1*, *T15D6.5*, *ins-8*, and *ins-20*. Real-time PCR results showed a significant increase in *ins-8* and *ins-20* expression in the OP+LK group, whereas there was no significant difference in the increased expression of *glct-3*, *T15D6.1*, and *T15D6.5* (Fig. 3).

Survival and motility evaluation of mutants

Focusing on *ins-20*, the expression of which showed greater elevation after feeding with *L. kimchii*, we performed a survival analysis and motility evaluation using *ins-20*-deficient mutants. Two alleles, *tm1947* and *tm5634*, of the *ins-20* mutant *C. elegans* were used in these experiments. Survival and motility analyses were performed in a similar manner as the first experiment. The results showed that in *tm1947*, the *L. kimchii* admixture did not affect the longevity of the nematodes but significantly mitigated the reduction in motility (Fig. 4A, 4B, 4G). In *tm5634*, the *L. kimchii* admixture had no effect on longevity or motility (Fig. 4C, 4D, 4G). Next, a survival analysis and motility evaluation were conducted using mutants deficient for *daf-2*, which encodes an insulin receptor. The *L. kimchii* admixture did not affect the longevity of the nematodes but significantly increased their motility (Fig. 4E, 4F).

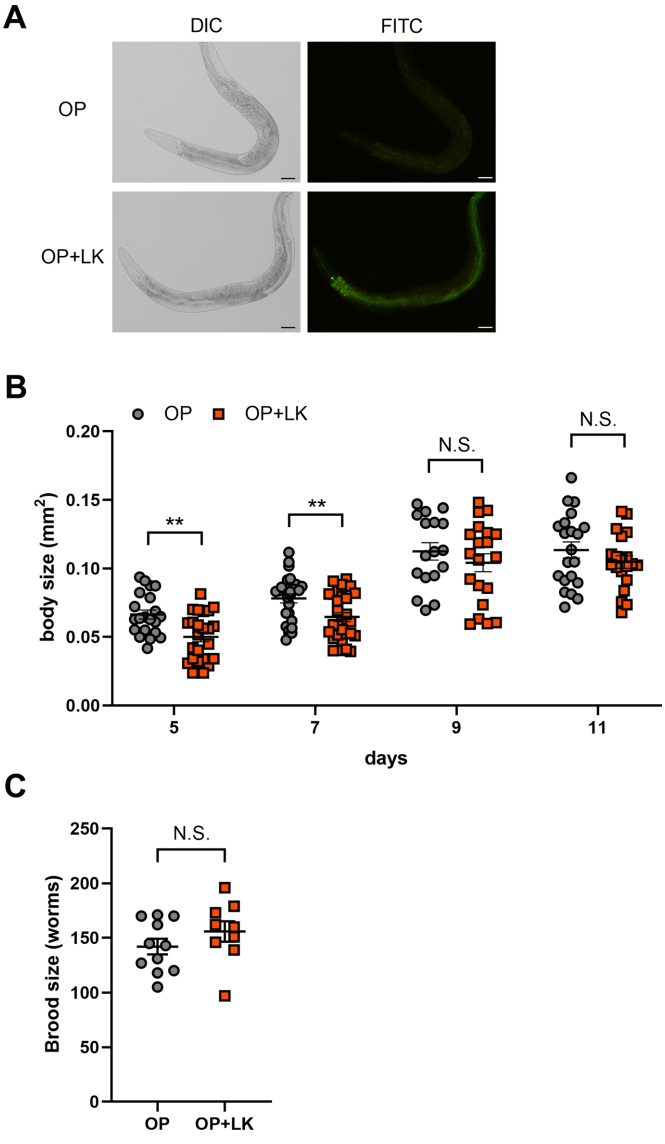

Fig. 2. Evaluation of *Lactococcus kimchii* (LK) ingestion by *C. elegans*. (A) Microscope images of *C. elegans* after feeding of OP alone or with a mixture of OP and FITC-labeled LK. Scale bars, 50 μm; DIC, differential interference contrast. (B) Body sizes of worms fed OP or OP+LK from days 5–11. (C) Comparison of brood size between worms fed OP (n=11) and those fed OP+LK (n=9). All values are presented as individual plots and means ± standard error of the mean (SEM). Statistical analysis was performed using unpaired two-tailed t-tests. **$p<0.01$. N.S., not significant.

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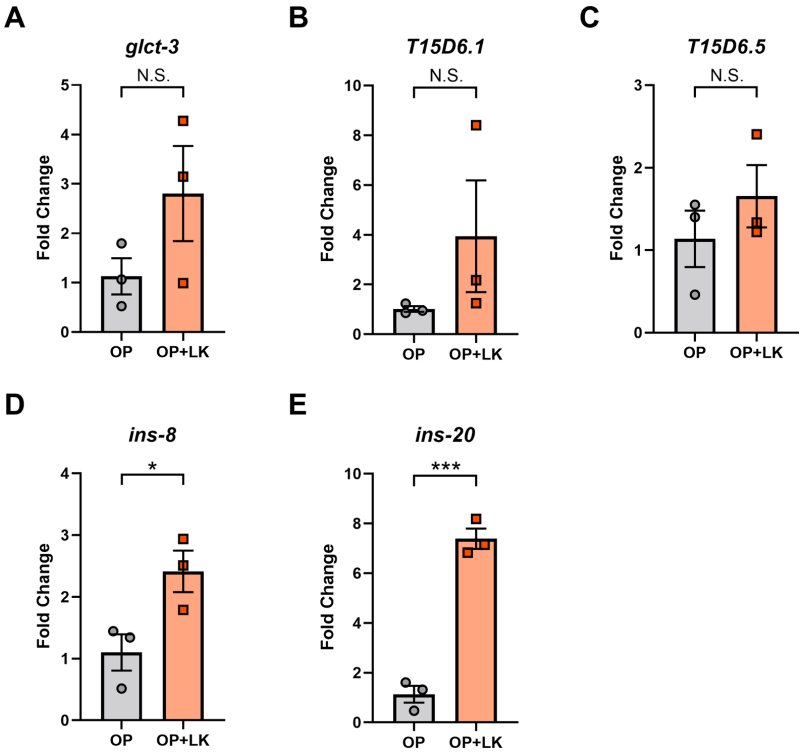

Fig. 3. Expression of genes related to top-ranked Gene Ontology (GO) terms quantified by real-time polymerase chain reaction (PCR). Expression of genes with biological functions enriched in the LK-fed group. Relative mRNA levels in animals fed OP+LK compared with those of the OP group. Values are presented as individual plots and means ± SEM of three independent experiments. Statistical analyses were performed using an unpaired two-tailed t-test. *p<0.05; ***p<0.001.

DISCUSSION

This study assessed the health-promoting characteristics of *L. kimchii* employing *C. elegans* as a model organism and focused on examining the lifespan and motility of *C. elegans* when fed *L. kimchii*. Feeding nematodes with a mixture of OP and *L. kimchii* significantly extended their lifespan and alleviated age-related declines in motility. Dietary restriction can extend the lifespan of *C. elegans*, reduce increases in body length, and decrease the number of eggs laid [29, 30]. To investigate this possibility, the intake of *L. kimchii* and the brood and body sizes of the worms were measured. FITC-labeled *L. kimchii* was detected in *C. elegans* fed *L. kimchii*, suggesting that the nematodes ingested *L. kimchii*. There was a minimal difference in body size and no difference in brood size between the OP+LK and OP groups. These results suggest that the longer lifespan and enhanced motility observed in the nematodes result from consuming *L. kimchii*, rather than from dietary restriction. Although the lifespan of adult nematodes fed *L. lactis* subsp. *lactis* NBRC 100933 (type strain) was not significantly increased compared with that in a group fed OP50, their locomotion was significantly improved [31]. This suggests that the longevity effects on nematodes differ among *Lactococcus* spp. Notably, the age dependency of the improved locomotion caused by feeding of *L. kimchii* remains unclear. Further experiments are needed to determine whether *L. kimchii* increases locomotion in young nematodes.

We also analyzed the expression of genes upregulated by *L. kimchii* feeding; GO analysis identified five genes (*glct-3*, *T15D6.1*, *T15D6.5*, *ins-8*, and *ins-20*) with significantly enriched biological functions. The GO terms for the enriched genes included chondroitin sulfate proteoglycan, proteoglycan, glycoprotein biosynthetic and metabolic processes, and regulation of signaling receptor activity (Supplementary Table 3). Furthermore, the expression of *ins-20* showed the greatest upregulation. We subsequently evaluated survival and locomotor performance using two alleles in *ins-20*-deficient mutants (*tm1947* and *tm5634*). The results showed that the *ins-20*-deficient mutant lost the effects of lifespan extension and mitigation of motility reduction caused by *L. kimchii* feeding. Notably, these effects were both abolished in *tm5634*, whereas the effect on lifespan extension, but not on locomotion, was eliminated in *tm1947*. This may be because *tm5634* has large deletions in the coding region and further upstream [32], which eliminate normal *ins-20* expression and the phenotypes observed for both lifespan and motility. In contrast, *tm1947* has no deletions in the coding region but has a deletion in the upstream promoter region [32], suggesting that *ins-20* expression is decreased but not abolished, resulting in a weak phenotype. These results suggest that *ins-20* is involved in lifespan extension and mitigation of motility loss in *C. elegans* fed *L. kimchii*.

ins-20 encodes for an insulin-like peptide and an agonist of insulin receptors [33]. *C. elegans* insulin-like peptides are expressed in various tissues depending on the gene, including in neurons, the epidermis, and the gut, and *ins-20* is expressed in the sensory head neuron [34]. The insulin/IGF-1 signaling (IIS) pathway is integral to aging [35], and *Clostridium butyricum* MIYAIRI 588 [36] and *Pediococcus acidilactici* CECT 9879 (pA1c) [37] extend the lifespan of *C. elegans* through the IIS

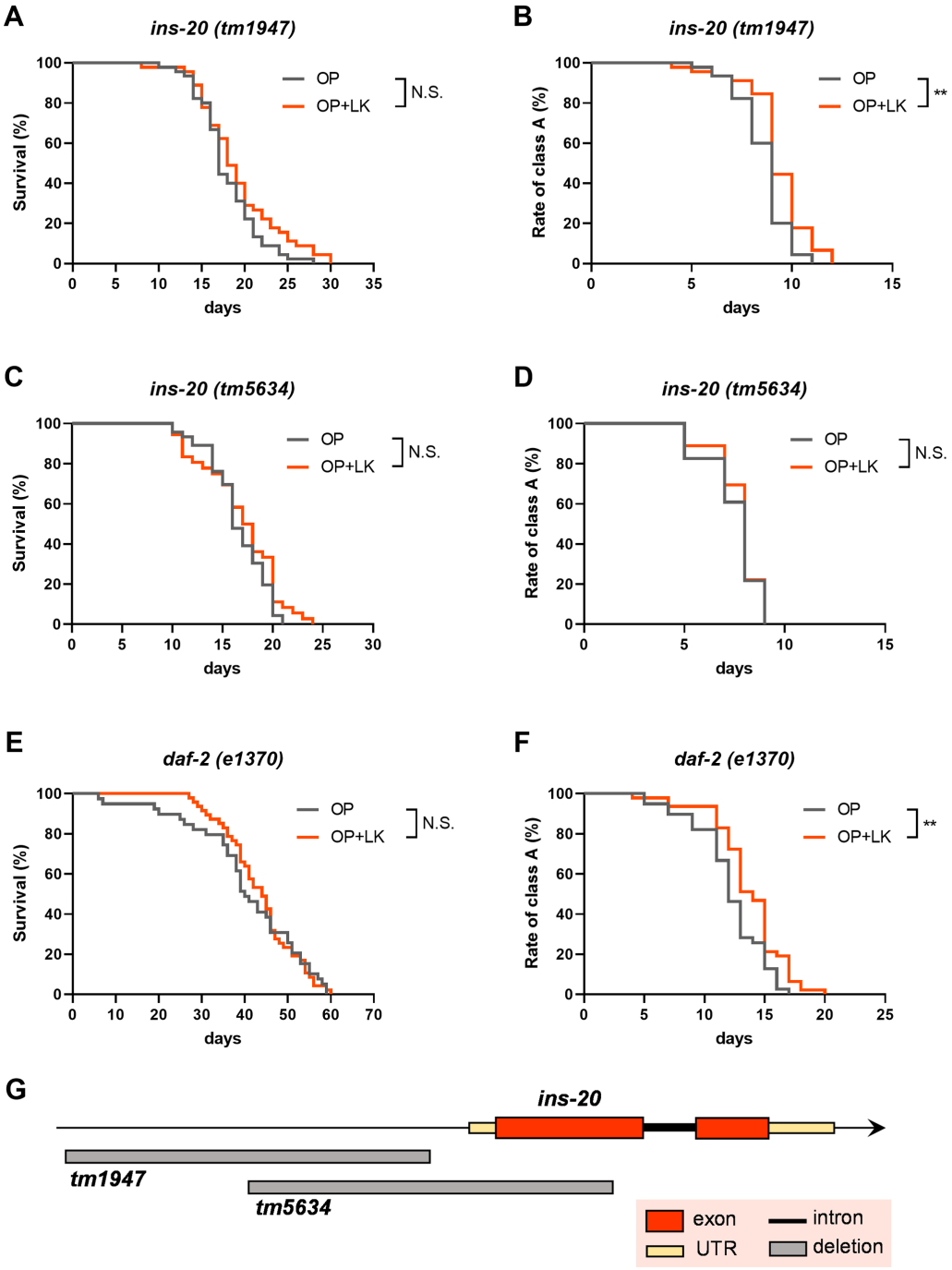

Fig. 4. Survival and healthspan curves of *ins-20* or *daf-2* mutant *C. elegans* fed OP or OP+LK. (A, B) Curves of the *ins-20* (*tm1947*) mutant (OP, n=45; OP+LK, n=45). (C, D) Curves of the *ins-20* (*tm5634*) mutant (OP, n=46; OP+LK, n=36). (E, F) Curves of the *daf-2* (*e1370*) mutant (OP, n=39; OP+LK, n=47). (G) The genomic structure of *ins-20* and mutation sites of deletion mutants are shown. Differences in survival were tested for significance using the log-rank test. ***p<0.001; **p<0.01. N.S., not significant. Detailed lifespan data and statistics are provided in Supplementary Table 6. Detailed healthspan data and statistics are provided in Supplementary Table 7.

signaling pathway. We hypothesized that the IIS pathway is involved in extending the lifespan and mitigating the motility loss of *C. elegans*; hence, we evaluated survival and motility in *C. elegans* lacking the insulin receptor gene *daf-2*. The results showed that *daf-2* mutant nematodes lost the extension of lifespan but not the mitigation of reduced locomotor activity. The mean and maximum rates of motility enhancement were higher in N2 (14.7% and 20.1%, respectively) than in *daf-2* mutants (7.8% and 5.9%, respectively). Thus, the IIS pathway may be partially responsible for the effects of *L. kimchii* on nematode lifespan extension and the mitigation of motility loss. However, we cannot rule out the possibility that *L. kimchii* could not further extend the lifespan of the long-lived *daf-2* mutants.

Our study showed that *L. kimchii* extends the lifespan of *C. elegans* and alleviates declines in locomotor performance, with the insulin signaling pathway potentially involved in this process.

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The specific and comprehensive involvement of the insulin signaling pathway in the lifespan extension and improvement of locomotion induced by *L. kimchii* could not be determined from the genetic analysis in the current study. The functions of the insulin peptide, the insulin receptor and its downstream kinase cascade, and transcription factors should be clarified through epistasis analysis using loss/gain-of-function mutants of the genes encoding the respective factors, biochemical experiments to detect phosphorylation, and imaging that would reveal nuclear transfer of transcription factors. Nevertheless, we showed for the first time the role of the insulin-like peptide INS-20 in the health effects, including lifespan extension and motility improvement, of lactic acid bacteria. Our research indicates that *L. kimchii* exhibits potential as a probiotic/postbiotic with novel target actions, making it a promising candidate for the formulation of innovative health products.

AUTHOR CONTRIBUTIONS

Conceptualization, S.T. and E.K.-N.; investigation, S.T.; statistics, S.T. Y.T.; writing – original draft preparation, S.T., M.S.A., and Y.T.; writing – review and editing, S.T., M.S.A., Y.T., and E.K.-N; supervision, E.K.-N. All authors have read and approved the manuscript for publication.

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DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available in the Gene Expression Omnibus (GEO; GSE245679).

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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