Original Article

Streptococcus thermophilus extends lifespan through activation of DAF-16-mediated antioxidant pathway in *Caenorhabditis elegans*

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Streptococcus thermophilus **bacteria, which are widely used as fermented starter for dairy production, exert various beneficial health effects. Nevertheless, even though pro-longevity effects of various probiotics have been reported, no report has described** ***Streptococcus thermophilus*** **effects on longevity. This study was conducted to evaluate** ***Streptococcus thermophilus*** **effects on lifespan extension and to elucidate the** ***Streptococcus thermophilus*****-mediated longevity mechanism using** ***Caenorhabditis elegans*** **worms as a model animal. They were fed standard food (*Escherichia coli* OP50) or** ***Streptococcus thermophilus*** **from the young adult stage. Feeding with** ***Streptococcus thermophilus*****, compared to** ***Escherichia coli*** **OP50, to** ***Caenorhabditis elegans*** **extend the lifespan, reduced lipofuscin accumulation, and maintain vigorous locomotion. Feeding with** ***Streptococcus thermophilus*** **did not alter the worm growth curve or the offspring number, indicating that the** ***Streptococcus thermophilus*****-mediated lifespan extension is not attributable to caloric restriction. The qRT-PCR data showed that** ***Streptococcus thermophilus*** **increased the expression of** ***daf-16*** **and some of its downstream antioxidant genes. Furthermore, the pro-longevity effects of** ***Streptococcus thermophilus*** **were decreased in loss-of-function mutant of** ***daf-16*****. Results show that** ***Streptococcus thermophilus*** **extends the lifespan of** ***Caenorhabditis elegans*** **through DAF-16-mediated antioxidant pathway activation.**

Key Words**:** ***Caenorhabditis elegans*****, hydrogen peroxide, lactic acid bacteria, longevity, oxidative stress**

Probiotics, defined as living organisms which, when ingested in adequate amounts, confer a health benefit on the host, have been used for almost a century for the management of various medical disorders.[1] Several lines of evidence indicate that probiotics have various physiological effects on their host intestine, such as decreasing the colonization of pathogenic bacteria and regulating the mucosal immune response.[2] As systemic effects, probiotic bacteria can prevent metabolic disorders such as obesity and type 2 diabetes by reducing serum cholesterol and lipids.[3] Additionally, adequate intake of probiotics has been reported to extend the lifespan of various organisms.[4–6]

Streptococcus thermophilus (*S. thermophilus*), which are Gram positive bacteria used widely as traditional fermented starters for yogurt and cheese production, have positive health effects on the host.[7] Knowledge has accumulated to elucidate their beneficial effects against diseases such as chronic gastritis,[8] antibiotic-associated diarrhea,[9] lactose intolerance,[10] and colorectal tumorigenesis.[11] Moreover, some promising antioxidant activities of *S. thermophilus* have been observed from both *in vitro* and *in vivo* models.[12,13] Nevertheless, even though longevity effects of various probiotics have been explained in the literature, no report has described the influence of *S. thermophilus* on longevity.

Caenorhabditis elegans (*C. elegans*), a bacteriophagous soil nematode, has been used extensively as an experimental system for biological studies because of its morphological simplicity, transparent body, ease of cultivation, and amenability to genetic analysis. Moreover, the short and reproducible lifespan of *C. elegans* is very suitable for aging studies. The nematode lifespan can be influenced by genetic and environmental factors, including food factors. Genes involved in lifespan regulation are related to several evolutionarily conserved pathways that regulate aging processes, such as insulin/insulin-like growth factor-1.[14–16] Therefore, *C. elegans* represents a suitable model organism for evaluation of the impacts of nutritional stimuli and food factors on pro-longevity. In fact, some probiotics (such as lactic acid bacteria), compared with a standard food (such as *Escherichia coli*), extend the lifespan of *C. elegans*.[4,17,18]

Given this context, we evaluated whether *S. thermophilus* can extend the *C. elegans* lifespan. Additionally, we elucidated the mechanism underlying *S. thermophilus*-mediated lifespan extension in *C. elegans* using loss-of-function mutants.

Materials and Methods

Bacteria strains and culture conditions. *Escherichia coli* OP50 (OP50), used as the standard feed, was grown in Luria–Bertani broth for 48 h at 37°C. Two strains of *S. thermophilus* isolated from fermented milk product were cultured using de Man–Rogosa–Sharpe broth in an anaerobic condition for 48 h at 37°C. Strains T-1 (ST-T1) and 510 (ST-510) were obtained respectively from Kyoto Prefectural Technology Center for Small and Medium Enterprises (Kyoto, Japan) and Japan Dairy Industry Association (Tokyo, Japan). After the OP50, ST-T1, and ST-510 were collected by centrifugation and washed twice with S basal buffer [100 mM NaCl, 50 mM potassium phosphate (pH 6.0)], the bacteria were diluted to a final concentration of 10 mg (wet weight)/ml in S basal buffer.

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***C. elegans* strains and culture conditions.** The wild-type *C. elegans* strain Bristol N2 and its derivative mutant strains were provided by the Caenorhabditis Genetics Center (CGC; Minneapolis, MN). The loss-of-function mutants used for this study were CF1038: *abnormal Dauer formation* (*daf*)-*16* (*mu86*) and TK22: *mev-1* (*kn1*). Worms were maintained on nematode growth medium (NGM) plates seeded with OP50 at 20°C.[19] For synchronization, the worms were cultured on fresh NGM plates for 2–3 generations without starvation. The young adult worms were cleaned and collected, then broken up using a lysis solution (0.6% sodium hypochlorite, 200 mM sodium hydroxide). After 12–14 h, the isolated eggs were hatched. Synchronized L1 larvae were obtained.

Determination of *C. elegans* lifespan. The synchronized L1 larvae were fed with OP50 and grown to young adults. Then 5-fluorodeoxyuridine (0.5 mg/ml) was added to prevent progeny production.[20] The resultant synchronized hermaphrodites were transferred to NGM plates covered with OP50, ST-T1, or ST-510 (10 worms per 35 mm plate). After the plates were incubated, the numbers of live or dead worms were scored three times a week. Worms were inferred as "dead" when they failed to respond to a gentle touch with a worm picker. Worms that crawled off the plate and showed non-natural death, such as internal hatching or adhering to the plate wall, were regarded as lost and were not included in the analysis. Experiments were performed at least in triplicate. More than 82 worms for each group were used in the longevity assay.

Measurements of body and brood sizes. Four-day-old young adult worms were placed on NGM plates (one worm per 35 mm plate) seeded with OP50, ST-T1, or ST-510. For body size measurement, the body sizes of the live worms were measured every 24 h until 7 days of age. Images of the worms were taken using a True Chrome II^{+} camera (Fuzhou Tucsen Photonics Co., Ltd., Fujian, China) and were analyzed using Image J software (National Institutes of Health, Bethesda, MD). For this experiment, the area of a worm's projection was estimated automatically and was used as an index of body size. To measure the brood size, the parental worms were transferred every 24 h to fresh NGM plates until the end of the reproductive period. The resulting progenies were left to develop for 3 days; then the number of progeny was ascertained.[21]

Lipofuscin accumulation. The autofluorescence of intestinal lipofuscin was measured for use as an index of senescence. Four-day-old young adult worms were placed on NGM plates seeded with OP50, ST-T1, or ST-510 until they became 14-day-old adult worm. Randomly selected worms were washed with S basal buffer for 30 min and were then placed onto a 3% agar pad coated with 1 M sodium azide to induce anesthesia. Lipofuscin autofluorescence images were detected with excitation at 357 nm and emission at 447 nm using an imaging system (EVOS M7000; Thermo Fisher Scientific Inc., Waltham, MA). To calculate the lipofuscin-positive area, densitometry measurements were taken using Image J software.

Locomotory scoring. Four-day-old young adult worms were placed on NGM plates (a worm per 35 mm plate) seeded with OP50, ST-T1, or ST-510. The locomotory assay of worms was performed every 48 h until 18 days of age using a scoring method described in an earlier report.[22] Worms were classified according to a four-point scale: Class "a" worms showed spontaneous movement or vigorous locomotion in response to prodding. Class "b" worms did not move unless prodded or appeared to have uncoordinated movement. Class "c" worms moved only their head and/or tail in response to prodding. Class "d" worms were dead.

Quantitative real-time PCR. Four-day-old young adult worms were placed on NGM plates seeded with OP50, ST-T1, or ST-510 for 24 h. Worms were collected and washed with S basal buffer for 30 min. Total RNA was then extracted and reverse-transcribed. The resultant cDNA was subjected to quantitative real-time PCR (qRT-PCR) using each specific primer described in Table 1. PCR was performed using a PowerUP SYBR Green PCR Master Mix and a real-time PCR system (StepOnePlus; Applied Biosystems, Forster, CA). The PCR conditions were denaturation at 95°C for 15 s, primer-annealing and elongation at 60°C for 1 min, with subsequent melting curve analysis during which the temperature was increased from 60°C to 95°C. The Ct values were transformed into relative quantification data using the $2^{-\Delta\Delta Ct}$ method. Data were normalized to the *act-1* endogenous control.

Fluorescent straining of H_2O_2 in the living worms. To detect cellular H_2O_2 levels in worms, fluorescent probe BES-H_2O_2-Ac (Fujifilm Wako Pure Chemical Corp., Tokyo, Japan) was used. For use as a staining solution, the BES-H_2O_2-Ac was diluted to a final concentration of 200 μM using S basal buffer. Four-day-old young adult worms were placed on NGM plates seeded with OP50, ST-T1, or ST-510 until they developed to 14-day-old adult worms. Five to ten worms were treated with 450 ml of the staining solution for 1 h. The worms were washed with S basal buffer for 30 min and were then mounted on a 3% agar pad with 1 M sodium azide. The H_2O_2 levels were observed with excitation at 470 nm and emission at 510 nm using the EVOS M7000 Imaging System.

Statistical analysis. *C. elegans* survival was calculated using the Kaplan–Meier method. Survival differences were tested

Table 1. Primers used for qRT-PCR analyses

Gene	Forward	Reverse
daf-2	5'-GCCCGAATGTTGTGAAAACT-3'	5'-CCAGTGCTTCTGAATCGTCA-3'
daf-16	5'-TCCTCATTCACTCCCGATTC-3'	5'-CCGGTATATTCATGAACGTG-3'
daf-12	5'-GTTCTGGTGAAGCCGAAGAG-3'	5'-AAGGGTGGTTGAGGTACGTG-3'
skn-1	5'-TCAGGACGTCAACAGCAGAC-3'	5'-CGTGGAGATTCCGAAGAGAG-3'
daf-7	5'-GTGCTGCTTGTATGACCTCG-3'	5'-GGTTTCCGCCAAGTTGAAGT-3'
sod-1	5'-CTGCCTGCGGTGTCATTG-3'	5'-GAGACGCGATTCAGGTAGTCACT-3'
sod-2	5'-AGGATCCACTTGAGGCAACAA-3'	5'-TGCTCCCAGACGTCAATTCC-3'
sod-3	5'-CCTGTGCAAACCAGGATCCT-3'	5'-CCCAAACGTCAATTCCAAAAA-3'
sod-4	5'-TTGGGACGCGGTACTTCAG-3'	5'-GCAAGTCGGCTTCCAGCAT-3'
sod-5	5'-GCCTCTTCGGAGCGAACA-3'	5'-TCTCGATCGACGTGGACAAC-3'
ctl-1	5'-GCCGGAGCCCATGGAT-3'	5'-CGGCCTTACAGTACTTGGTGATG-3'
ctl-2	5'-GGTCACCCATGACATCTCCAA-3'	5'-TGCTTCCCGACCTTGTTGA-3'
act-1	5'-CACGGTATCGTCACCAACTG-3'	5'-GCTTCAGTGAGGAGGACTGG-3'

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for significance using the log-rank test. In other experiments, the significance of comparisons between OP50, ST-T1, and ST-510 was determined using Student's *t* test. Statistical analyses were conducted using statistical software (BellCurve Excel–Toukei software; SSRI, Tokyo, Japan). All results are expressed as mean ± SE. Differences for which $*p<0.05$ were inferred as significance.

Results

***S. thermophilus* extend the lifespan of *C. elegans* without the participation of caloric restriction.** To assess the effects of *S. thermophilus* on the *C. elegans* lifespan, worms were fed ST-T1 or ST-510 from an age of 5 days. As presented in Fig. 1, the lifespans of the worms fed ST-T1 or ST-510 were significantly longer than those of worms fed the standard OP50. The survival rates were similar among the three groups until day 8. After day 11, the two groups fed *S. thermophilus* showed different survival curves compared to those of the worms fed OP50.

Caloric restriction can extend the lifespan of various organisms including *C. elegans*. The caloric-restricted worms exhibit small body and brood sizes.[23] To elucidate whether the ST-T1-mediated or ST-510-mediated lifespan extensions resulted from caloric restriction, the body and brood sizes of worms fed ST-T1 or ST-510 were compared with those of control worms fed OP50. Feeding with ST-T1 or ST-510 did not alter the worm growth curve (Fig. 2A). Similar results were obtained when the brood size was determined (Fig. 2B). These results indicate that ST-T1 or ST-510 extended the lifespan of *C. elegans* irrespective of caloric restriction effects.

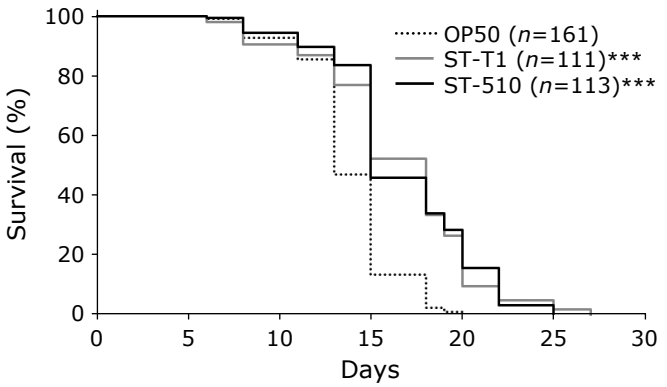

Fig. 1. Effects of *S. thermophilus* on the *C. elegans* lifespan. The synchronized L1 larvae were fed with OP50 until the young adult stage. The resultant worms were transferred to a fresh NGM plate seeded with OP50 or *S. thermophilus*. Two strains were used: *S. thermophilus* strain T-1 (ST-T1) and *S. thermophilus* strain 510 (ST-510). The numbers of live and dead worms were scored three times a week (n = 111–161 worms/group). Data were calculated using the Kaplan–Meier method. Survival differences were tested for significance using the log-rank test. Mean lifespans ± SE were as follows: OP50, 13.8 ± 0.2 days; ST-T1, 16.5 ± 0.4 days; ST-510, 16.7 ± 0.3 days. Significant differences relative to OP50 (***$p<0.001$) are shown.

Effects of *S. thermophilus* on age-related biomarkers in *C. elegans*. Lipofuscin accumulation and muscle function are known to correlate with aging processes in *C. elegans*.[24] Lipofuscin is a lipid peroxidation product and its accumulation is determined by autofluorescence. The autofluorescence intensities in worms fed ST-T1 or ST-510 were decreased significantly compared with that of worms fed OP50 (Fig. 3A and B).

Locomotive capability was assayed as an indicator of muscle function. The locomotory score was evaluated every 48 h until 18 days of age, as described in the Materials and Methods. During the experimental period, the proportion of worms displaying vigorous locomotion (class a) was always higher in ST-T1-fed or ST-510-fed worms than in OP50-fed worms (Fig. 3C).

DAF-16-mediated antioxidant pathway involvement in *S. thermophilus*-mediated longevity. The insulin/insulin-like growth factor-1-mediated signaling (IIS) pathway and transforming growth factor-β (TGF-β) pathway contribute to lifespan extension in *C. elegans*.[25] To investigate whether these two pathways are involved in the pro-longevity effects of *S. thermophilus*, the expression of these genes related to lifespan extension was determined using qRT-PCR. As portrayed in Fig. 4A, the expression levels of the *daf-16*, *daf-12*, and *daf-7* were higher in ST-T1-fed or ST-510-fed worms than in OP50-fed worms. Results show that *skinhead-1* (*skn-1*) expression was significantly lower in ST-T1-fed or ST-510-fed worms. Feeding with ST-510, but not with ST-T1, suppressed *daf-2* expression.

To elucidate whether the ST-T1-mediated or ST-510-mediated lifespan extension is related to enhanced *daf-16* expression, the lifespan of *daf-16* mutant worms fed ST-T1 or ST-510 was compared with that of *daf-16* mutant worms fed OP50. The lifespans of the worms fed ST-T1 or ST-510 were significantly longer than those of worms fed the standard OP50. The ST-T1-mediated or ST-510-mediated elongation rates in wild type worms was 18.9% or 20.4%, whereas that in *daf-16* mutant worms was 5.3% or 6.5%. These results suggest that the ST-T1-mediated or ST-510-mediated lifespan extension is dependent to some degree on the DAF-16 signaling pathway.

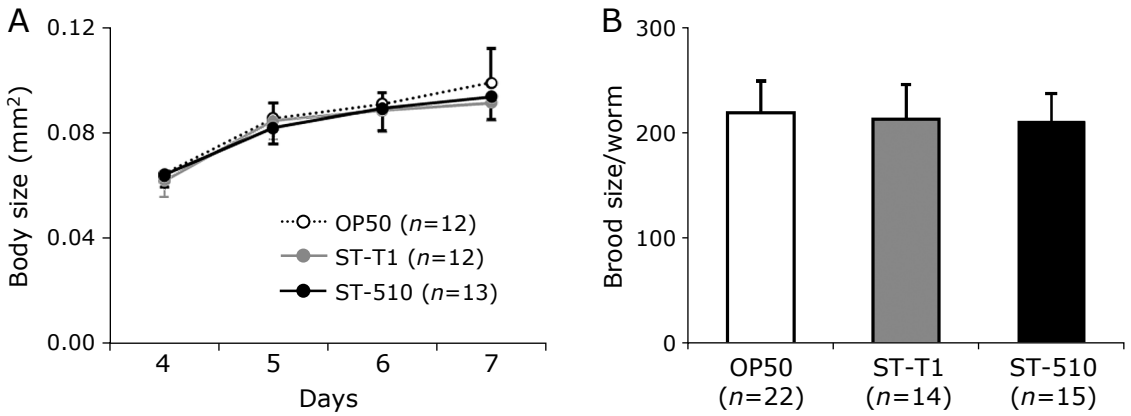

Fig. 2. Effects of *S. thermophilus* on body and brood sizes of *C. elegans*. Young adult worms were transferred to NGM plates seeded with OP50 or *S. thermophilus* (ST-T1 or ST-510): (A) body size was determined with 12–13 worms for each bacterial strain; and (B) brood size was determined with 14–22 worms for each bacterial strain. Data represent the mean ± SE.

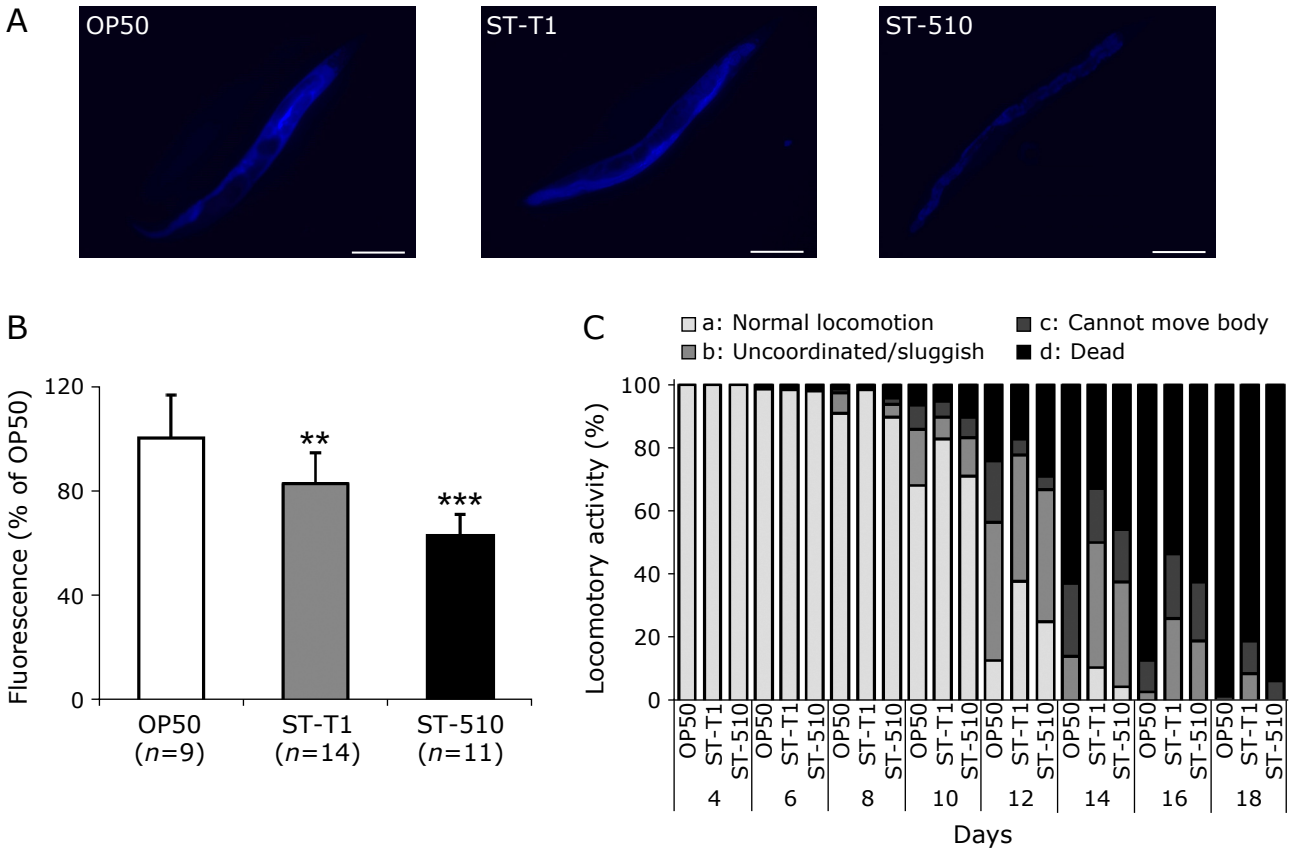

Fig. 3. Effects of *S. thermophilus* on lipofuscin accumulation and locomotive activity of *C. elegans*. Young adult worms were transferred to NGM plates seeded with OP50 or *S. thermophilus* (ST-T1 or ST-510). (A) At 14 days old, lipofuscin was measured by assessing autofluorescence using an EVOS M7000 Imaging System. Representative images of worms fed each bacterial strain are shown. Scale bar = 200 μm. (B) Autofluorescence of lipofuscin was quantified using Image J software. Data represent the mean ± SE (n = 9–14). Significant differences relative to OP50 (**p<0.01 and ***p<0.001) are shown. (C) At 4–18 days old, the worms were assigned to four classes based on locomotion: Class "a" worms showed spontaneous movement or vigorous locomotion in response to prodding. Class "b" worms did not move unless prodded or appeared to have uncoordinated movement. Class "c" worms moved only their head and/or tail in response to prodding. Class "d" worms were dead. The bars represent the proportion of each class at the indicated time period.

To investigate whether the DAF-16 signaling contributes to the ST-T1-mediated or ST-510-mediated lifespan extension further, we ascertained the expression levels of antioxidant genes located downstream of DAF-16.[26] As presented in Fig. 5A, the expression levels of *superoxide dismutase* (*sod*)-*3*, *sod-4*, *sod-5*, *catalase* (*ctl*)-*1*, and *ctl-2* were higher in ST-T1-fed or ST-510-fed worms than in OP50-fed worms. Both *sod-1* and *sod-2* expressions were significantly lower in ST-T1-fed or ST-510-fed worms. When the worms fed with the respective bacteria were treated with BES-H_2O_2-Ac as a fluorescent probe for detecting H_2O_2, the intestinal tract and its neighboring tissues were stained significantly in the OP50-fed worms, but not in the ST-T1-fed or ST-510-fed worms (Fig. 5B). Furthermore, we investigated the effect of ST-T1 or ST-510 on the lifespan of *mev-1* mutant worms, an oxidative stress hypersensitive strain.[27] They were compared with the lifespan of *mev-1* mutant worms fed *E. coli* OP50. As presented in Fig. 5C, the lifespans of the worms fed ST-T1 or ST-510 were significantly longer than those of worms fed the standard OP50. These data suggest that DAF-16-mediated antioxidant pathway is involved in the ST-T1-mediated and ST-510-mediated longevity.

Discussion

During the *C. elegans* aging process, the worms change biomarkers of aging such as lipofuscin and locomotory activity.[24] Results obtained in this study demonstrate that feeding the two strains of *S. thermophilus* (ST-T1 and ST-510), compared to OP50, to *C. elegans* extends the lifespan, reduces lipofuscin accumulation, and maintains vigorous locomotion. Additionally, DAF-16-mediated antioxidant pathway is involved in the *S. thermophilus*-mediated longevity.

Caloric restriction is recognized as a method to extend lifespan not only in numerous non-mammalian taxa but also in mammals, including primates.[23,28] Results of the present study show that feeding with *S. thermophilus* did not alter the worm growth curve or offspring number, indicating that caloric restriction is not involved in the *S. thermophilus*-mediated lifespan extension in *C. elegans*. Accumulated evidence suggests that probiotics of various type, including lactic acid bacteria, extend the *C. elegans* lifespan independently of the effects of caloric restriction. Kwon *et al.*[29] reported that *Propionibacterium freudenreichii*, a candidate non-lactic acid probiotic used in the fermentation of Swiss Emmental cheese, extends the lifespan of *C. elegans* via activation of its innate immune system. A recent study using a *C. elegans* model demonstrated the pro-longevity effect of *Lactobacillus fermentum* strain JDFM216.[18] It is particularly interesting that this strain attached actively to the worm intestine and stimulated host defenses through nuclear hormone receptor-related transcriptions. Additionally, Donato *et al.*[30] found that biofilm-proficient *Bacillus subtilis* colonized the *C. elegans* intestine and extended the worm lifespan significantly

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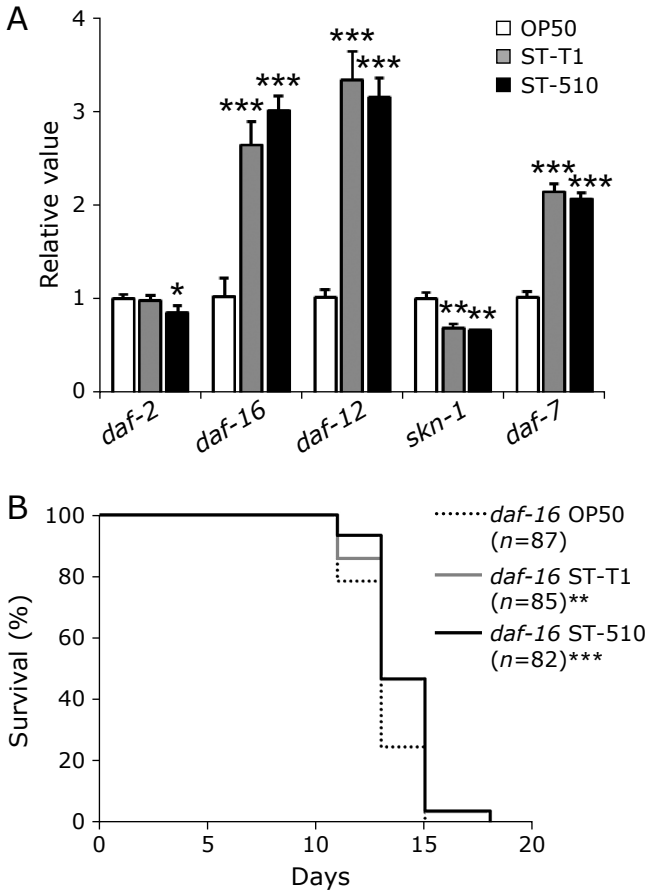

Fig. 4. Involvement of DAF-16 in *S. thermophilus*-mediated lifespan extension. (A) Young adult worms were transferred to NGM plates seeded with OP50 or *S. thermophilus* (ST-T1 or ST-510) for 24 h. The RNA was extracted and subjected to qRT-PCR. Then the expression levels of genes related to lifespan extension were evaluated. Data were normalized to the level of *act-1*. Data represent the mean ± SE ($n = 3$). Significant differences relative to OP50 (*p<0.05, **p<0.01, and ***p<0.001) are shown. (B) Young adult stage of loss-of-function mutant of *daf-16* were fed OP50 or *S. thermophiles* ($n = 82$–87 worms/group). The Kaplan–Meier survival curve is depicted. Mean lifespans ± SE were as follows: OP50, 13.1 ± 0.1 days; ST-T1, 13.8 ± 0.2 days; ST-510, 13.9 ± 0.2 days. Significant differences relative to OP50 (**p<0.01 and ***p<0.001) are shown.

longer than did biofilm-deficient isogenic strains. The pro-longevity effect of the *Bacillus subtilis* biofilms depended on DAF-2/DAF-16 signaling. To elucidate the molecular mechanism of *S. thermophilus*-mediated lifespan extension, it may be necessary to verify whether *S. thermophilus* have biofilm-forming activity and intestinal colonization.

It is particularly interesting that some favorable antioxidant activities of *S. thermophilus* have been observed from both *in vitro* and *in vivo* models.[7] Ito *et al.*[13] reported that feeding with *S. thermophilus* strain YIT2001 caused a decrease in lipid peroxidation in the mouse colonic mucosa. The underlying mechanisms of the antioxidant activities remain unknown, but they can be related to the activity of antioxidant enzymes produced by *S. thermophilus*, such as SOD.[31] Our data showed that lipofuscin accumulation in worms fed *S. thermophilus* was significantly lower than that in worms fed OP50. Furthermore, results of qRT-PCR analyses revealed that feeding with *S. thermophilus* upregulated *daf-16* expression. The pro-longevity effects of *S. thermophilus* were slight in *daf-16* mutant worms. DAF-16, an orthologue of the forkhead box-containing protein O subfamily (FoxO) transcription factor, acts in IIS pathway that regulates longevity under the conditions of oxidative stress and caloric restriction. In fact, in the worms fed *S. thermophilus*, the expression of several antioxidant genes was increased. The accumulation of H_2O_2 was suppressed. Earlier reports have described that the expressions of *sod-3*, *ctl-1*, and *ctl-2* were positively regulated by DAF-16, whereas those of *sod-1* and *sod-2* were negatively regulated by DAF-16.[26,32,33] Actually, *sod-4* and *sod-5* are putative target genes of DAF-16.[34,35] These data suggest that DAF-16-mediated antioxidant pathway is involved in the *S. thermophilus*-mediated lifespan extension. This assertion is supported by the data of *mev-1* mutant worms.

The expression and activity of DAF-16 are regulated tightly by several signaling pathways. Two well-known pathways are the IIS and TGF-β pathways. The former is initiated by the binding of insulin-like peptides to the receptor DAF-2; the DAF-2-mediated signaling negatively regulates DAF-16 function.[36] By contrast, the TGF-β pathway is related to innate immunity, body morphology, and longevity. DAF-7 is the TGF-β-related ligand for the regulation of longevity.[37] The qRT-PCR data obtained from the present study indicate that expression levels of *daf-7* and *daf-12* are increased in worms fed *S. thermophilus*. No drastic change was observed in the expression of *daf-2*. The expression of *skn-1*, an orthologue of nuclear factor-erythroid-related transcription factor, is negatively regulated by DAF-12.[38] This finding is consistent with our obtained data. In addition, DAF-12, which is a putative target gene of DAF-7, is a well-known nuclear hormone receptor. It affects the innate immune system by inducing the production of antimicrobial peptides.[39] Several reports have indicated that DAF-7 and DAF-12 positively regulate DAF-16 function.[40,41] Based on those findings, one might infer that *S. thermophilus*-mediated *daf-16* upregulation is related to the activation of DAF-7/TGF-β pathway, but not of IIS.

In conclusion, results presented herein suggest that *S. thermophilus* ingestion enhances DAF-16-mediated antioxidant system through activation of DAF-7/TGF-β pathway. It then delays *C. elegans* aging processes and extends their lifespan. Although our data represent results only of this nematode model, DAF-16-mediated antioxidant system and TGF-β pathway are universal in regulating aging. They are conserved among living organisms, including primates.[25] Therefore, the mechanisms identified in this study may apply to other species, including *Homo sapiens*. To elucidate the detailed molecular mechanisms of *S. thermophilus*-mediated lifespan extension, further investigations must be conducted using some loss-of-function mutants such as DAF-12 defective worms. However, our data have revealed a part of the beneficial effect of *S. thermophiles* for our health.

Author Contributions

The respective roles of the authors are the following: ND, NI, YN, and YH conceived the project and prepared the manuscript; ND, CO, HN, KY, and TB performed the experiments; YK cultivated and maintained the microorganisms. All authors critically reviewed the manuscript. YN and YH supervised all aspects of the study.

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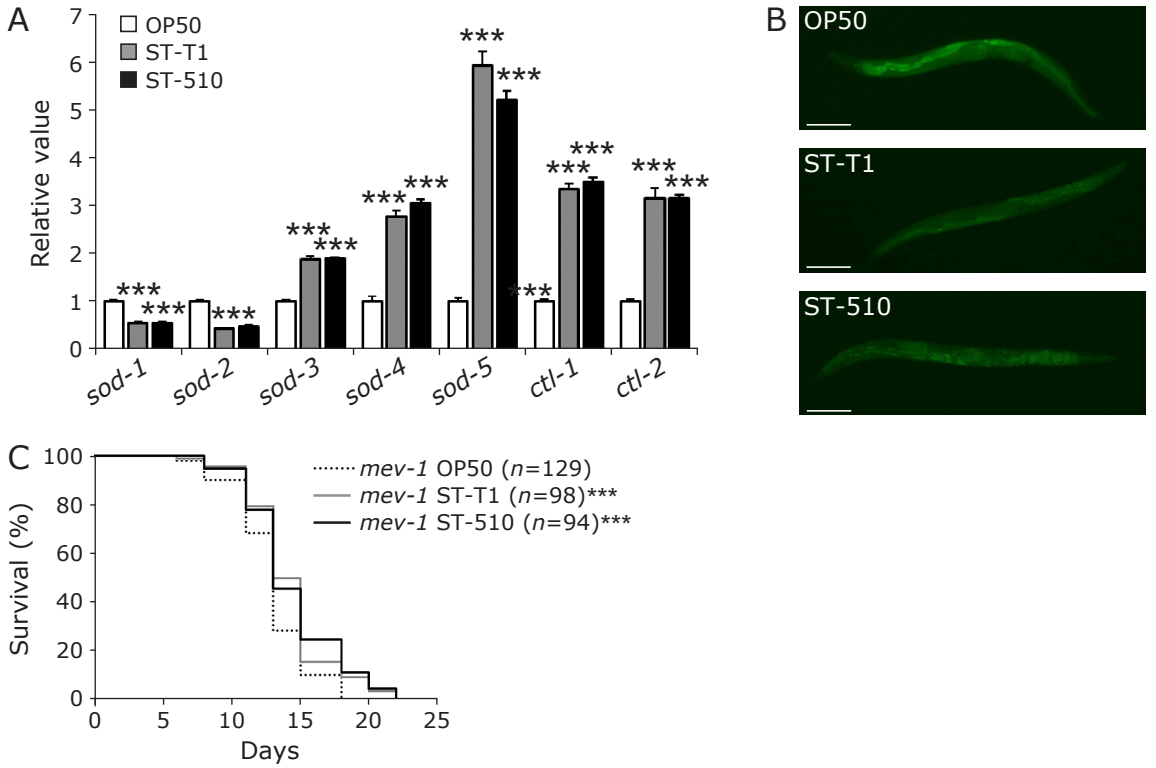

Fig. 5. Involvement of antioxidant pathway in *S. thermophilus*-mediated lifespan extension. (A) Young adult worms were transferred to NGM plates seeded with OP50 or *S. thermophilus* (ST-T1 or ST-510) for 24 h. The RNA was extracted and subjected to qRT-PCR. Then the expression levels of genes related to antioxidant pathway were evaluated. Data were normalized to the level of *act-1*. Data represent the mean ± SE (n = 3). Significant differences relative to OP50 (***p<0.001) are shown. (B) At 14 days old, accumulation of H_2O_2 was detected using the fluorescent probe BES-H_2O_2-Ac with an EVOS M7000 Imaging System. Representative images of worms fed each bacterial strain are shown. Scale bar = 100 μm. (C) Young adult stage of loss-of-function mutant of *mev-1* were fed OP50 or *S. thermophilus* (n = 94–129 worms/group). The Kaplan–Meier survival curve is depicted. Mean lifespans ± SE were as follows: OP50, 12.9 ± 0.2 days; ST-T1, 14.1 ± 0.3 days; ST-510, 14.4 ± 0.3 days. Significant differences relative to OP50 (***p<0.001) are shown.

to Yuji Naito (No. 16824414) from the Ministry of Agriculture, Forestry and Fisheries of Japan.

Abbreviations

C. elegans	*Caenorhabditis elegans*
ctl	catalase
daf	abnormal Dauer formation
FoxO	forkhead box-containing protein O subfamily
IIS	insulin/insulin-like growth factor-1-mediated signaling
NGM	nematode growth medium
OP50	*Escherichia coli* OP50
qRT-PCR	quantitative real-time PCR
skn	skinhead
sod	superoxide dismutase
S. thermophilus	*Streptococcus thermophilus*
TGF-β	transforming growth factor-β

Conflict of Interest

YK is a salaried employee of the Mill Souhonsha Company Limited. This does not alter our adherence to JCBN policies on sharing data and materials. YN received scholarship funds from EA Pharma Co. Ltd. and Taiyo Kagaku Co., Ltd.; a collaboration research funds from Taiyo Kagaku Co., Ltd. and Mill Souhonsha Co. Ltd.; and received lecture fees by Mylan EPD Co., Takeda Pharmaceutical Co. Ltd., Mochida Pharmaceutical Co. Ltd., EA Pharma Co. Ltd., Otsuka Pharmaceutical Co. Ltd., and Miyarisan Pharmaceutical Co. Ltd. The present research was partly supported by these funds. Neither the funding agency nor any outside organization has participated in the study design or have any competing interests. These companies had final approval of the manuscript. The other authors have no conflict of interest to disclose.

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