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Latilactobacillus sakei improves resistance to oxidative stress in *Caenorhabditis elegans* by activating the UPR^{mt} through the HAF-1/ATFS-1 mitochondrial import efficiency regulator

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ABSTRACT: It seems an attractive research direction for microorganisms to show the repair effect on host organisms during host aging. Although previous reports have pointed out the benefits of probiotics in restoring host mitochondrial health, few studies have focused on the important role of mitochondrial unfolded protein response (UPR^{mt}). In this study, we demonstrated that feeding *Latilactobacillus sakei* ZFM232 (LS232) can enhance the resistance of *Caenorhabditis elegans* (*C. elegans*) to different environmental stresses. In addition, we found that LS232 intake increased the cellular respiration rate of nematodes at different stages of aging and effectively prevented mitochondrial aging caused by nematodes as they age. Compared with normal mitochondria, LS232 enhances intestinal mitochondria activity during nematodes' aging process, effectively increasing the mitochondrial membrane potential level at different stages of aging and reducing calcium ion permeability. Through genetic screening, we found that the expression of UPR^{mt}-related genes was significantly upregulated. Then, UPR^{mt} transcriptional activation was demonstrated by mutants' lifespan verification and fluorescent expression of *hsp-6* and *hsp-60*::GFP. In addition, we found that LS232 intake promoted the transfer of the transcription factor ATFS-1 to the nucleus and its expression in the intestine to regulate UPR^{mt}. RNAi interference revealed that the upstream gene *haf-1* was also crucial for expressing UPR^{mt}. Finally, we demonstrated that LS232 extends its lifespan by activating UPR^{mt} mainly through HAF-1/ATFS-1 in response to mitochondrial damage repair stress. Our findings elucidate the health benefits of LS232 to the host and its potential application in strategies to improve mitochondrial function.

Keywords: *Lactobacillus sakei*; Oxidative stress; Mitochondrial repair; *Caenorhabditis elegans*; HAF-1/ATFS-1; UPR^{mt}

1. Introduction

Oxidative damage has been identified as a primary catalyst for mitochondrial dysfunction, a hallmark of aging, and numerous diseases[1]. In recent years, our research has investigated how oxidative damage impacts mitochondrial homeostasis and the body's mechanisms for preserving mitochondrial function, including the antioxidant defense system and mitochondrial autophagy. Maintaining mitochondrial homeostasis is imperative for cellular health, particularly during aging, when mitochondrial function alterations directly influence the body's physiological state. Therefore, the integrity of mitochondrial function necessitates

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cooperation and communication with other cellular compartments[2-3]. The maintenance of protein homeostasis is contingent on multiple interrelated cellular stress pathways, including the heat shock response (HSR), oxidative stress (OS), and the unfolded protein response (UPR). Among these, UPR^{mt} has been demonstrated to be closely associated with promoting healthy aging and maintaining mitochondrial homeostasis. Consequently, exploring the mechanism of UPR^{mt} is imperative for the body to adapt to metabolic and environmental stress[4-5].

UPR^{mt} has been identified as an important defense against mitochondrial protein toxic stress in mammals, *Drosophila*, and *C. elegans*[6]. In a pathway named after the unfolded protein response of the endoplasmic reticulum, UPR^{mt} senses protein toxic stress in mitochondria and programs gene expression to restore organellar protein homeostasis. Like UPR^{ER}, UPR^{mt} upregulates target genes, including organelle-specific chaperones and proteases[7]. The process of UPR^{mt} is primarily mediated by the transcription factor ATFS-1. Oxidative phosphorylation disturbances, excessive reactive oxygen species (ROS), impaired complex assembly, and protein misfolding have been shown to impair the efficiency of mitochondrial protein import and activate the stress-related transcription factor ATFS-1[8-9]. ATFS-1 is imported into healthy mitochondria via its mitochondrial targeting sequence (MT S), where it is degraded. In instances where mitochondrial import efficiency is compromised, ATFS-1 is translocated to the nuclear localization sequence (NLS), where it activates UPR^{mt} [10-11]. ATF5, the mammalian orthologue of ATFS-1, has been demonstrated to regulate UPR^{mt} in mammalian cells and upregulate transcription of numerous *C. elegans* quality control genes in response to mitochondrial stress[12]. In addition, UPR^{mt} signals to the nucleus via the mitochondrial peptide export protein HAF-1, further promoting ATFS-1-mediated mitochondrial defense gene expression and activating innate immune factors, including lysozyme and antimicrobial peptides[13]. Recent studies have shown that the mitochondrial chaperone HSP-60 plays a key role in triggering the UPR^{mt} in mitochondrial stress regulation, further highlighting the importance of the UPR^{mt} in maintaining mitochondrial health[14].

In 1907, Dr. Metchnikoff introduced the concept of probiotics, defined as “microorganisms that are beneficial to the human body after consumption.” He theorized that these microorganisms were instrumental in promoting human health and longevity, a hypothesis that has since prompted substantial research. These studies have reported a variety of beneficial effects associated with probiotics, including antimicrobial activity, protection of the intestines, and nutrient supplementation[15-16]. It is crucial that once the foodborne microorganisms colonize the host intestine, they can exert their effects and continue to provide benefits. *Lactobacillus plantarum* NJAU-01 has been demonstrated to mitigate subacute aging in mice induced by D-galactose[17]. *Latilactobacillus sakei* has unique features that distinguish it from other probiotic strains. It was originally isolated from fermented foods such as sake and meat products, and because it produces bacteriocins and flavor compounds, it is often used in the food industry as a starter and protective culture. Its remarkable adaptability to different environments suggests a strong colonization potential in the host gut. Unlike many common lactobacilli, *Latilactobacillus sakei* also possesses a distinct genomic profile. In mouse

models, GABA produced by *Latilactobacillus sakei* was associated with enhanced long-term memory recovery, and when combined with exercise, GABA significantly reduced weight gain, thereby acting as an anti-obesity agent[18]. Bajpai et al. observed that the exopolysaccharides produced by *Latilactobacillus sakei* exhibited strong superoxide dismutase-like activity and effectively inhibited α-glucosidase[19]. They also showed concentration-dependent tyrosinase activity and high polyphenol content, indicating potent antioxidant properties. Compared with other strains, LS232 has significant anti-aging activity, but its potential to regulate mitochondrial stress repair and aging-related pathways has not been thoroughly studied. Therefore, this study aims to explore the anti-aging effects of *Latilactobacillus sakei* 232, providing a theoretical basis for further research on the probiotic functions of this bacterium.

C. elegans serves as a preeminent model organism for studying aging and regulating intestinal probiotics[20-21]. This organism's attributes, including its short lifespan, ease of genetic manipulation, and the high degree of conservation of its signaling pathways with mammals, make it an invaluable model. In this model, multiple signaling pathways have been revealed to play pivotal roles in aging and immune regulation, including the p38/MAPK pathway, the insulin signaling pathway, and the UPR^{mt}-mediated mitochondrial stress response. These findings contribute to a more profound comprehension of the mitochondrial unfolded protein response and offer novel insights for future research on applying probiotics in anti-aging and health regulation.

This study aimed to evaluate whether LS232 can delay oxidative damage and aging in *C. elegans* by improving stress resistance and mitochondrial function. Our results show that long-term intake of LS232 activates the UPR^{mt}, and this process dependent on the transcription factor ATFS-1 and its upstream regulator *haf-1*. We propose that LS232 promotes the nuclear translocation and intestinal expression of ATFS-1, thereby maintaining cellular homeostasis and repairing mitochondrial health in a mitochondria-dependent manner. Overall, these findings highlight the potential of LS232 as a probiotic candidate for treating mitochondrial diseases characterized by oxidative stress.

2. Materials and methods

2.1 Preparation of Latilactobacillus sakei cells

LS232 was isolated from fresh milk from a dairy farm in Hangzhou City, Zhejiang Province, China. The strain was identified using the BLAST tool based on a basic local alignment search in the NCBI database and is deposited at the China Typical Culture Collection Center (CCTCC M, accession number: 2025928). LS232 (1%) was inoculated into MRS medium and incubated at 37℃ for 12 hours. Then, LS232 cells were collected by centrifugation at 5000 r/min for 10 min at 4℃ and washed twice with PBS. The washed LS232 cells were subjected to OD detection to ensure that the values obtained in the experiment were consistent with those of OP50. Next, under the condition of ensuring normal growth of *C. elegans*, the concentrations of LS232 and OP50 were finally maintained at 1×10^9 CFU/mL.

2.2 Strains and culture conditions

The following strains were utilized in this study: N2 (Bristol, wild type), SJ4143 zcIs17[*ges-1*::GFP(mit)], SJ4100 zcIs13[*hsp-6p*::GFP+lin-15], SJ4058 zcIs9 [*hsp-60*::GFP+lin-15], SJ17 zcIs4 [*hsp-4*::GFP] V. *xbp-1(zc12)*, VC3201 *atfs-1(gk3094)* V, OP675 wgIs675 [*atfs-1*::TY1::EGFP::3Xflag+unc-119] were procured from the Cryptic Nematode Genetics Center (CGC) at the University of Minnesota; we backcrossed the mutant strain and the N2 wild-type nematodes used in this experiment a total of three times before the experiment. *E. coli* OP50 was obtained from the laboratory of Professor Wu at Huazhong University of Science and Technology (Wuhan, China). 5-fluoro-uracil (FUDR) and the essential raw materials required for the preparation of the nematode growth medium (NGM) were procured from Sigma-Aldrich. It is noteworthy that all the purchased reagents met the requisite standards of quality, with purity levels ranging from analytical grade to superior.

2.3 Assessment of UV damage resistance

Using wild-type nematodes at the first day of the L4 stage after synchronization and transfer them to NGM plates containing FUDR and different bacterial foods and grow them in an incubator at 20 ℃. For the worms, change the medium and food every day. Following a five-day culture, the nematodes were collected and transferred to NGM plates devoid of bacterial contamination. Thereafter, they were exposed to ultraviolet (UV) radiation at a dose of 1200 J/m^2. The plates were then placed in an incubator set to 20℃, and the mortality of the nematodes was recorded at one-day intervals until all nematodes had perished. The plates were replaced every other day[22]. The nematodes were gently touched with a platinum wire to observe the extent of death and to exclude abnormal nematodes every day. All experiments were performed in triplicate, with each group containing over 60 nematodes.

2.4 Measurement of oxidative stress resistance

The test for oxidative stress was based on previous studies with some modifications [23-24]. Reversal effects were gauged by subjecting synchronized L4 wild-type nematodes to a stressor. Using wild-type nematodes at the first day of the L4 stage after synchronization and transfer them to NGM plates containing FUDR and different bacterial foods and grow them in an incubator at 20 ℃. For the worms, change the medium and food every day. Following a five-day culture, the nematodes were picked and transferred to fresh NGM plates containing 200 μmol/L juglone (transferred after 30 minutes of exposure). The nematodes were then counted every hour until they all died. At each count, the nematodes were gently touched with a platinum wire to observe the extent of death and to exclude abnormal nematodes. It is important to note that all experiments were performed in triplicate, with more than 60 nematodes used in each group.

2.5 OCR-based measurement of cellular metabolic status

The experiment was carried out as previously described, with modifications[25]. As mentioned above, synchronized wild-type nematodes that reach the first day of stage L4 are used, transferred to NGM plates containing FUDR and different bacterial foods, and grown in an incubator at 20 ℃. For the worms, change the medium and food every day. After five and ten days of growth on different bacterial plates, the worms were

collected, and the samples were quickly frozen in liquid nitrogen. An OCR^- based assay kit (Dojindo, Kumamoto, Japan) was utilized to estimate cellular oxygen consumption by measuring the level of extracellular oxygen based on the phosphorescence signal of an oxygen-sensitive probe quenched under aerobic conditions. A working solution was prepared by adding the oxygen probe to the BPSE according to the manufacturer's instructions. Following a 24-hour storage period in EGTA-AM, the samples were subjected to a centrifugation process at 4 ℃ for a duration of 5 minutes. Thereafter, the sample was suspended in 100μL of the corresponding working solution and mixed and incubated. The fluorescence intensity was subsequently measured utilizing a Safire® microplate reader (Tecan, Mannedorf, Switzerland) at wavelengths of 500 nm and 650 nm, respectively, and the emission wavelength.

2.6 Morphological observation of intestinal mitochondria

The intestine of *C. elegans* is rich in mitochondria and is the main place responsible for energy metabolism and homeostasis regulation mediated by antioxidant stress. Consequently, the assessment of mitochondrial damage in *C. elegans* is significantly influenced by intestinal mitochondrial function. Transgenic nematodes *ges-1*::GFP (mit) were placed on NGM plates containing OP50 or LS232, respectively, and cultivated in a medium at 20 ℃. After synchronous feeding for five days, the worms were washed five times with M9 buffer to remove residual bacteria. The worms were then anesthetized and fixed, after which the integrity of the muscle mitochondria was observed under a fluorescence microscope (Olympus TH4-200). Images were collected, and the average pixel intensity of each worm was determined using ImageJ software. Thirty worms were used for each treatment, and all experiments were conducted in triplicate.

2.7 Mitochondrial calcium concentration detection

The experiment was carried out as previously described, with modifications based on the sample material[26]. In brief, nematodes were placed on NGM plates containing OP50 or LS232, respectively, and grown in a 20 ℃ medium. After synchronous feeding for three, five, and ten days, the nematodes were washed repeatedly with M9 buffer and collected in a centrifuge tube. The calcium ion level was measured using the Fluo-4AM kit (Beyotime, China) according to the instructions. 1 mL of dyeing solution was added to each centrifuge tube and incubated at 37 ℃ for 30 minutes in the dark. After incubation, the remaining dye was washed off with M9 buffer. Following the anesthetization and fixation of the nematodes, the fluorescence intensity was measured under a fluorescence microscope (Olympus TH4-200) using an excitation wavelength of 490 nm and an emission wavelength of 525 nm. The nematodes were then divided into different time periods for dynamic photography, and the average pixel intensity of each worm was measured using ImageJ software. The data were then normalized. Thirty nematodes were used for each treatment, and all experiments were conducted in triplicate.

2.8 JC-1 Staining

As with the method described above, the nematodes were collected in a centrifuge tube after being washed repeatedly with M9 buffer five and ten days after the first feeding at the L4 stage. To permeabilize the

nematode cuticle for staining, the nematodes were suspended in PBS containing 2% paraformaldehyde. The samples were then gently agitated at room temperature for 30 minutes and washed with isopropanol. Subsequently, the samples were transferred to microtubes containing JC-1 staining solution and incubated in the dark for 30 minutes in an incubator. The fluorescent area of the mitochondria in the worms was observed under a fluorescence microscope (Olympus TH4-200). The red (excitation 550 nm, emission 600 nm) and green (excitation 485 nm, emission 535 nm) fluorescence emissions were measured, and images were captured[24]. The average pixel intensity of each worm was determined using ImageJ software. Thirty nematodes were used for each treatment, and all experiments were conducted in triplicate.

2.9 RT-qPCR for mRNA quantification

Prior to quantification, total RNA was isolated using Trizol reagent for real-time (RT) quantitative polymerase chain reaction (qPCR) assays. cDNA was produced using the Quantscript RT kit (Accurate Biology, China). As mentioned above, nematodes at the L4 stage of synchronization were selected and grown at 20 ℃ on NGM medium. RT-qPCR reactions were performed using the Light-Cycler system (Roche Applied Science) and qPCR Supermix (Qiagen), with *act-1* as the reference gene, using the $2^{-\Delta\Delta Ct}$ method[27].

2.10 Mutant lifespan determination

C. elegans were placed on NGM plates containing OP50 and cultivated in 20℃ incubators. After a specified period, the nematodes were washed off with M9 buffer and synchronized with a bleach solution to obtain synchronized nematodes. Wild-type nematodes at the time of reaching the first day of the L4 phase after synchronization were selected and placed on NGM plates containing FUDR (50 μmol/L) and different bacteria for growth. The experimenters initiated the counting on the first day of L4 transfer, and animals were transferred to fresh NGM medium daily and monitored for the number of live animals until death unless otherwise stated. Survival was observed at predetermined times, and worms were transferred to new NGM plates with a pick or control plate to ensure adequate food until no worms survived. It should be noted that data analysis did not include nematodes that had burrowed into the medium or escaped from it[28]. The lifespan calculation software Graph Pad was used for statistical analysis, and log-rank lifespan analysis was performed to analyze the mean lifespan and maximum lifespan. Notably, all experiments were performed in triplicate, with a minimum of 80 nematodes utilized in each group.

2.11 RNAi interference

RNA interference (RNAi) is a biotechnology that can inhibit the expression of specific genes. Gene interference experiments were performed on SJ4100 (*hsp-6*::*gfp*)nematodes by introducing the L4440 plasmid and the target gene. The L4440 empty vector was introduced into OP50 HT115 containing ampicillin (100 mg/mL^{-1}) by positive cloning. L1 stage nematodes were then fed OP50 HT115 containing the target gene. After being cultured to the L4 larval stage, RT-qPCR was used to analyze the expression of the target gene *haf-1*. Then, the synchronized nematodes were then transferred to plates containing a mixture of OP50 HT115 (L4440-*haf-1*) and LS232, with a ratio of 1:1, and grown at 20 ℃ on NGM plates (30 μL per plate) [29-30].

2.12 ATFS-1::GFP nuclear localization assay

Nuclear localization experiments were performed using the transgenic nematode OP675. Briefly, eggs were incubated synchronously, and then the L4 stage worms were selected and placed on NGM plates containing FUDR (50 μmol/L) and different bacteria for five days. The worms were then transferred to slides containing 8% agarose and anesthetized with NaN_3 (20 μmol/L). The fluorescent area of the nematodes was observed and calculated using the GFP filter mode under a fluorescence microscope (Olympus TH4-200). Expression was carried out using ATFS-1::GFP, and the percentage of expression was calculated from the fluorescent emission area using ImageJ software[31-32]. The data is then normalized. Sixty nematodes were used for each treatment, and all experiments were conducted in triplicate.

2.13 Fluorescence imaging analysis

Fluorescence imaging analysis was performed using transgenic nematode strains SJ4058 and SJ4100. Briefly, the nematodes were transferred to a slide containing agarose and anesthetized with NaN_3 (20 μmol/L). The fluorescent area of the nematodes was then observed and calculated using a GFP filter mode under a fluorescence microscope (Olympus TH4-200). The percentage expression at each concentration is calculated based on the area of fluorescence emission. Data normalization is also performed. Thirty nematodes were used for each treatment, and the experiment was repeated three times.

2.14 Statistical analysis

Statistical analyses were performed using Graph Pad Prism 7.0 (San Diego, USA) and SPSS software 19.0 (Chicago, IL, USA). All results are presented as mean ± standard deviation (SD). Numerical estimation was performed using a significant analysis of variance (ANOVA) and the Waller-Duncan test in SPSS.

3. Results

3.1 Long-term ingestion of LS232 can significantly extend the lifespan of C. elegans in different environments

Oxidative damage to the body caused by the external environment is considered to be one of the main causes of cellular aging[33]. In many aging studies, extending the life span is associated with enhanced resistance to oxidative damage and other stresses. Consistent with many previous studies, we used two different stress methods to analyze the effect of LS232 feeding on the stress resistance of *C. elegans* under various conditions and to evaluate the range and threshold of stress responses produced *in vivo* when exposed to external stimuli. The survival rates of nematodes following ultraviolet stress and treatment with juglone are presented in Fig. 1 and Table 1. The results demonstrate that LS232-fed nematodes exhibit significantly prolonged lifespans under various stresses compared to the control group. Among them, the median lifespan of the LS232 treated group after exposure to UV damage was 7.51 ± 0.50, its tolerance increased by 20.1%. We treated *C. elegans* with OP50 and LS232 for about 5 days and then placed the nematodes on agar containing 200μmol/L juglone for testing (The early lethality of juglone is essentially because it is a strong oxidative stress inducer that can rapidly disrupts intracellular redox homeostasis and impairs mitochondrial function). Compared with the control group, the intake of LS232 can effectively reduce the cytotoxicity of

juglone on nematodes, and the median lifespan of the nematodes was 6.50 ± 0.50 which was 20.4% longer lifespan compared to the control. In addition, we found that the survival curve of all nematodes fed LS232 in nematodes exposed to UV or juglone treatment showed an overall shift to the right compared with nematodes treated with OP50. These results show that long-term feeding with LS232 can improve the body's ability to resist oxidative stress and extend its lifespan.

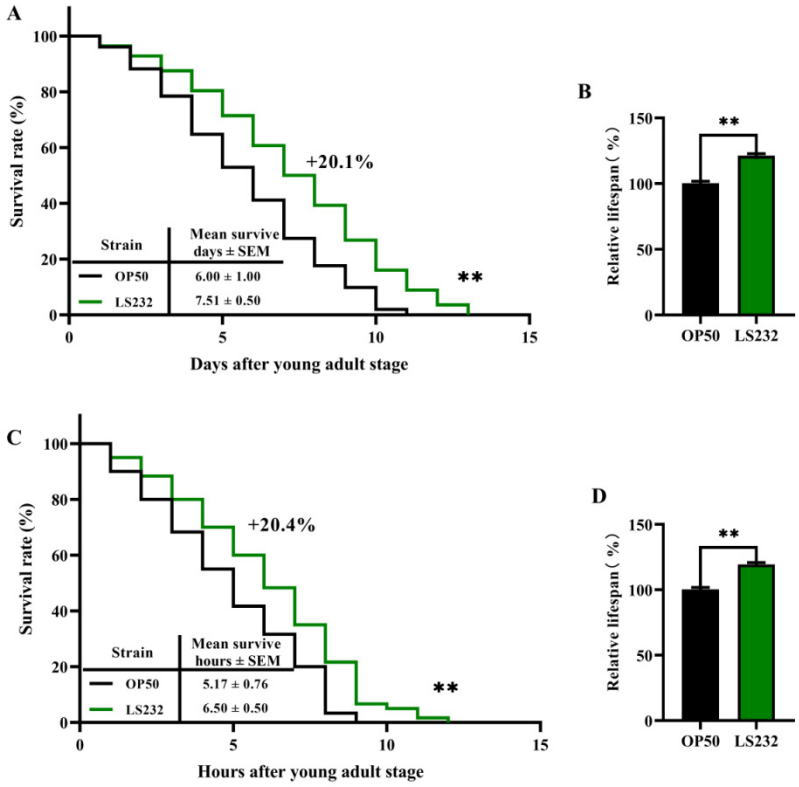

Fig. 1 Effects of LS232 on the resistance of *C. elegans* to different oxidative stress conditions. Fig. A shows the effect of feeding nematodes with different bacteria on their survival rate after exposure to ultraviolet radiation. The number of dead and living nematodes was counted every day until all the fed nematodes died. Fig. B shows the increase in the relative lifespan of the nematodes. Fig. C shows the effect of LS232 on nematode lifespan extension under the short-term action of juglone, where the number of dead and live nematodes was recorded every hour until all fed nematodes died. Fig. D shows the increase in relative nematode lifespan. Statistical analysis was performed using the log-rank (Mantel-Cox) test, and all tests were performed in triplicate. In all statistical analyses, values represent the mean ± standard error of three independent experiments, and $P < 0.05$ was considered statistically significant.

Table 1 Effect of different bacteria on the lifespan of *C. elegans* under different stressors

Injury mode	Group	N	Mean lifespan (h/days) ±SEM	% of control	Maximun lifespan(h/days)	*P* value
UV damage	OP50	51	6.00 ± 1.00	100.0	11	-
	LS232	56	7.51 ± 0.50	120.1	13	$P = 0.0020$
Juglone (200 μmol/L)	OP50	59	5.17 ± 0.76	100.0	9	-
	LS232	53	6.50 ± 0.50	120.4	12	$P = 0.0037$

Three replicates were carried out. Values with different letters in column are significantly different ($P < 0.05$). Significant differences were analyzed by log-rank (Mantel−Cox) tests.

3.2 Long-term intake of LS232 can enhance the reduction of respiratory function caused by aging.

Changes in the external environment have been demonstrated to induce cellular senescence in living organisms[21]. In this study, we used the perspective of cell respiration function in nematodes to demonstrate

whether the ingestion of LS232 can delay cell aging. We then used OCR determination to evaluate cells' functional and metabolic status because cellular respiration is a central process for maintaining cellular energy supply and physiological homeostasis, and the oxygen consumption of cells generally reflects the overall respiratory level and aging activity of cells. We measured the basal OCR^- level in cells of nematodes under normal growth conditions and dynamically evaluated it at different time points. The results showed that at different aging time points, compared with the control group, the OCR^- basal respiration rate of LS232 nematode cells gradually increased over time but decreased after the tenth day (Figs. 2A–C). Carbonyl cyanide-p-trifluoromethoxyphenone (FCCP) is a mitochondrial uncoupler that can traverse the inner mitochondrial membrane, thereby bringing protons from the intermembrane space back to the matrix side, uncoupling oxidative phosphorylation and inhibiting cellular respiration[34]. In this study, FCCP was utilized to induce interference with the aforementioned process. Remarkably, the respiratory function of LS232-treated cells did not appear to be significantly impaired. Furthermore, the OCR^- levels after FCCP exposure tended to be marginally elevated compared to the control group at various time points (Figs. 2D–F).

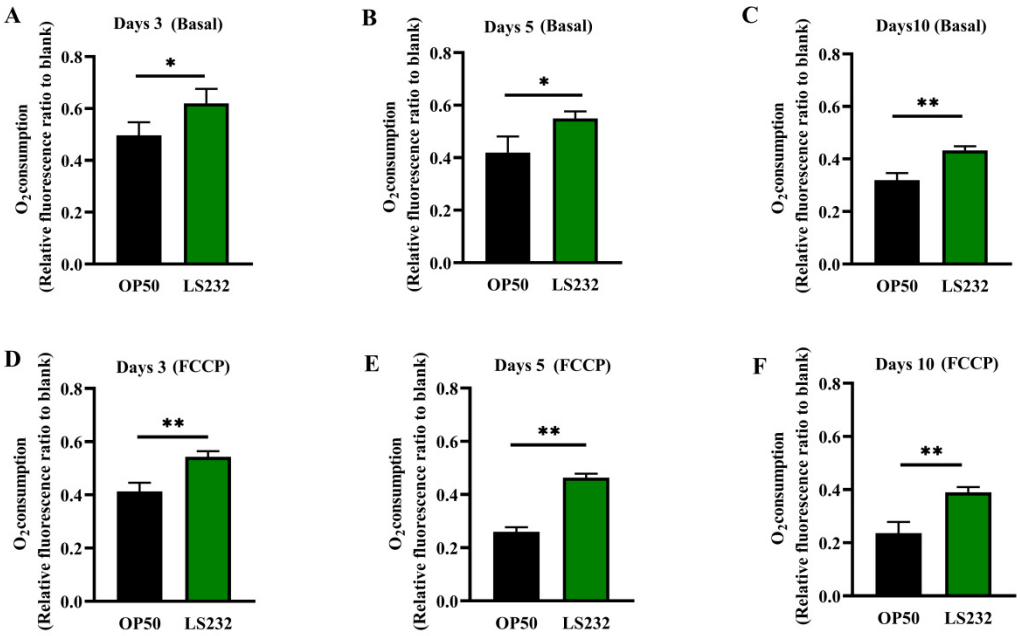

Fig. 2 Effect of chronic LS232 intake on improving the overall cellular respiration rate of *C. elegans*. Figs A-C illustrate the basal respiration rate of nematode cells at various time points following OP50 and LS232 intake. Subsequent figs (D–F) demonstrate the rescue effect of OP50 and LS232 intake on the basal respiration rate of nematode cells at various time points following FCCP intervention. The figures are marked as (∗) $P < 0.05$, (∗∗) $P < 0.01$, and (∗∗∗) $P < 0.001$. In all statistical analyses, the values are the mean ± standard error of three independent experiments.

3.3 Long-term ingestion of LS232 can enhance mitochondrial quality and restore mitochondrial function

Mitochondria are cells' energy generators, and their quality determines the metabolic rate of cells. They are closely related to oxidative stress in cells. We wanted to know whether ingesting LS232 would repair damaged mitochondria and increase their vitality. We used the *ges-1*::GFP (mit) transgenic nematode to observe the changes in intestinal muscle associated with aging. This type of nematode has evident mitochondrial expression in the intestinal region and is, therefore, used in studies related to muscle mitochondria. Following a 5-day feeding with OP50 and LS232, significant alterations in the intestinal

muscles and fiber tissue of *C. elegans* were observed in comparison to the control group (Fig. 3A). The intestinal musculature of the LS232-fed nematodes exhibited increased organization. In contrast, the control group displayed a fragmented and disordered state "aging state." Subsequently, the calcium ion (Ca^{2+}) concentration in the cells of the nematodes was measured. Ca^{2+} is essential for optimal mitochondrial function, but overload can damage mitochondrial function, leading to a decrease in mitochondrial inner membrane potential (ΔΨm) and ATP production, an increase in ROS release, and ultimately cell death[35]. An appropriate Ca^{2+} level in mitochondria helps maintain normal energy metabolism and cell function. Therefore, we chose different points in time to conduct the test. The results showed that with the passage of time and age-related accumulation of Ca^{2+} in mitochondria. However, compared with the control group, with the continued intake of LS232, the Ca^{2+} level in the mitochondria of nematodes gradually decreased and maintained a stable range (Figs. 3B-D). This also corresponds to the improved respiratory rate in the cells.

Mitochondrial membrane potential (ΔΨm) has been identified as a key indicator of mitochondrial function and cell health. Maintaining a stable ΔΨm is essential for the efficient operation of mitochondria, while a decrease in ΔΨm typically indicates impaired mitochondrial function, which may result in a decrease in energy metabolism, an increase in reactive oxygen species (ROS) levels, and subsequent induction of apoptosis or autophagy[24, 36]. We employed JC-1 staining to analyze the effect of LS232 feeding on the mitochondrial membrane potential of *C. elegans*. JC-1 exists predominantly as monomers in apoptotic cells, producing green fluorescence, while in non-apoptotic cells, mitochondria aggregate and emit red fluorescence. The results demonstrated that following approximately five days of OP50 and LS232 treatment, *C. elegans* fed with LS232 exhibited augmented red fluorescence and diminished green fluorescence (Fig. 3E), and the red/green fluorescence intensity ratio was significantly elevated (Fig. 3F), reaching approximately 1.5 times that of the control group. When the tenth day was reached, the beneficial effects of LS232 intake were still present. The red and green fluorescence intensity was about 0.5 times higher than that of the control group (Fig. 3H). This shows that even when the cells as a whole are gradually aging, LS232 intake can effectively improve mitochondrial quality, restore mitochondrial function, and thus prolong the healthy state of mitochondria in nematodes under different environmental conditions.

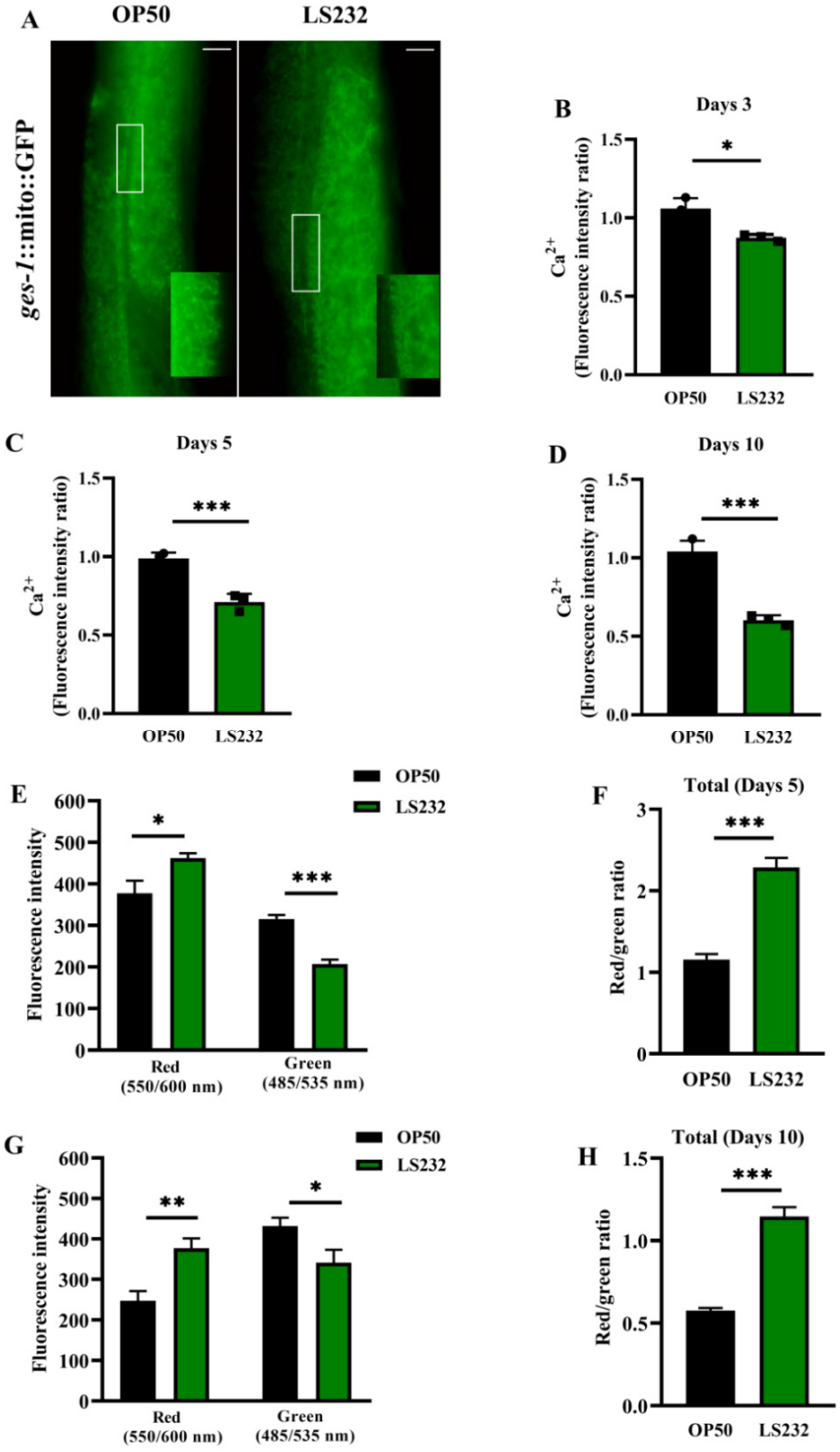

Fig. 3 Assessment of chronic LS232 exposure on mitochondrial mass and function in *C. elegans*. Fig. A shows representative images of LS232 feeding delaying the morphological changes of the intestinal mitochondrial network and enhancing the integrity of the nematode intestinal muscles. Figs. B-D demonstrate the alterations in intracellular Ca^{2+} levels following OP50 and LS232 feeding at various time points. Figs. E and G present the measurement of red or green fluorescence intensity by JC-1 staining after OP50 or LS232 feeding five and ten days. The Figs. F and H present the ratio of red/green fluorescence intensity, measured by JC-1 staining, for each group of nematodes subjected to different dietary regimens. The significance of the observed differences is indicated by the following marking system: (∗) $P < 0.05$, (∗∗) $P < 0.01$, (∗∗∗) $P < 0.001$. It should be noted that all statistical analyses reported here are based on the mean ± standard error of three independent experiments.

3.4 Long-term ingestion of LS232 can activate the mRNA expression of UPRmt-related genes in aging nematodes

Mitochondria, the energy centers of cells, are closely related to many important and conserved signaling pathways, including energy metabolism, signal transduction, oxidative stress response, and apoptosis[37]. UPR^{mt} is an evolutionarily conserved cellular stress response that is designed to maintain mitochondrial proteostasis and restore mitochondrial function. When the protein misfolding or accumulation in mitochondria leads to a protein homeostasis imbalance, UPR^{mt} is activated to enhance mitochondrial repair capacity by regulating the expression of nuclear genes. In light of the arguments above, an investigation was conducted into the impact of LS232 feeding on the expression levels of unfolded mitochondrial proteins and associated genes and signaling pathways in nematodes to further elucidate its potential mechanism. RT-qPCR analysis revealed that LS232 feeding can significantly affect the expression of related genes such as *hsp-6*, *hsp-60*, and *haf-1* in *C. elegans* (Fig. 4). These genes are pivotal components of the UPR^{mt} response that is activated by nematodes. Among these genes, the expression levels of *hsp-6* and *hsp-60* were found to be significantly increased, with an increase of approximately 13.76 ± 3.85 and 12.25 ± 1.94 times compared with the control group. Furthermore, the transcription factor *atfs-1* gene demonstrated elevated expression levels, exhibiting an upregulation of approximately 10.11 ± 1.96 times. Conversely, *hsp-4* exhibited no expression (Fig. 4D). Aging can lead to a decline in mitochondrial function, and an improvement in mitochondrial quality may be due to the LS232 activating UPR^{mt} to initiate repair processes.

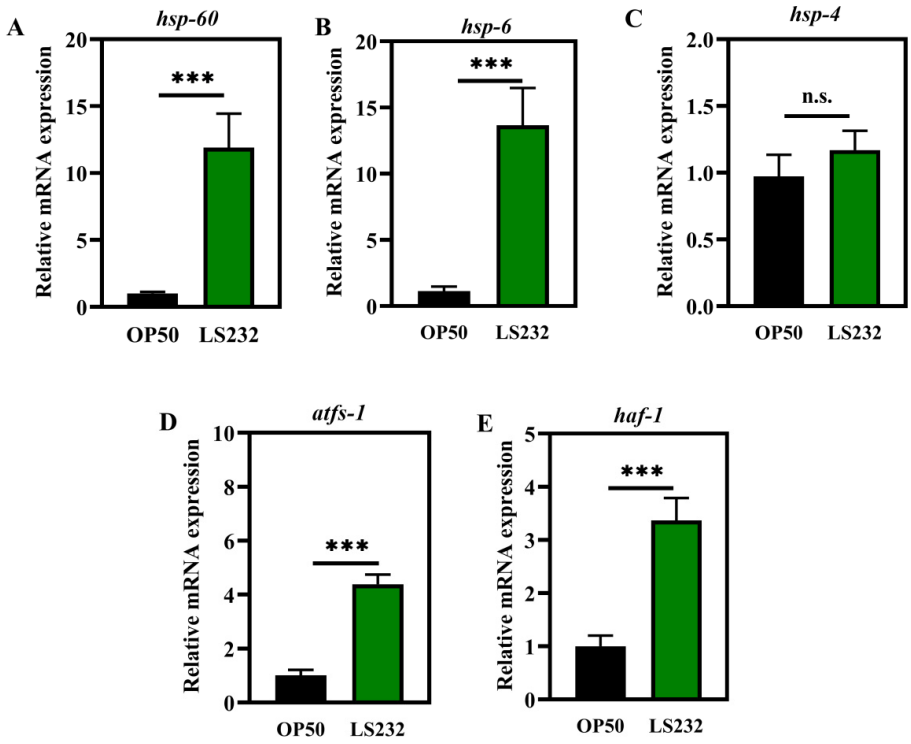

Fig. 4 The figure illustrates the long-term impact of LS232 on the mRNA expression of the nematode UPR^{mt}-related gene. The changes in mRNA expression of these genes and associated transcription factors following LS232 feeding were evaluated using RT-qPCR. The mRNA levels were normalized to those of *actin* as a control. The statistical significance of the observed differences is indicated by the following symbols: (∗) $P < 0.05$, (∗∗) $P < 0.01$, and (∗∗∗) $P < 0.001$. It is noteworthy that all statistical analyses were conducted on the mean ± standard error of three independent experiments.

3.5 LS232 extends the lifespan of C. elegans in different environments by activating UPR^{mt}

The UPR^{mt} has been identified as a pivotal protective mechanism employed by cells to address imbalances in mitochondrial protein homeostasis. The activation of molecular chaperones (e.g., *hsp-60* and *hsp-6*) by UPR^{mt} facilitates the restoration of mitochondrial function and the maintenance of protein stability. The mediation of UPR^{mt} is primarily orchestrated by the transcription factor ATFS-1. However, under conditions of stress or aging, the efficiency of ATFS-1 mitochondrial import is reduced, with part of ATFS-1 being transported to the nucleus, where it activates the expression of a series of genes related to mitochondrial protection, antioxidant stress, and immunity[39]. Through RT-qPCR results, we found that the intake of LS232 may activate the *atfs-1* gene. We used the UPR^{mt} mutant *atfs-1(gk3094)* and the UPR^{ER} mutant *xbp-1(zc12)* for lifespan assays to verify this finding. The results showed that the survival rate of LS232-fed *xbp-1(zc12)* mutants was increased compared with OP50-fed nematodes (Figs. 5C and D; Table 2). As *xbp-1(zc12)* is not a null mutation, this result indicates that UPR^{ER} is not activated. However, the lifespan of *atfs-1(gk3094)* mutants did not change significantly (Figs. 5A and B; Table 2), indicating that the transcription factor ATFS-1 is activated under continuous LS232 feeding. We then studied the fluorescent expression of two mitochondrial chaperone genes, *hsp-6*::GFP and *hsp-60*::GFP. These genes are necessary for the activation of UPR^{mt} in *C. elegans*. The expression of *hsp-6*::GFP has been observed to occur predominantly in the intestine and muscles, while *hsp-60*::GFP has been observed to occur in the mitochondria, with some expression in the cytoplasm. In the event of mitochondrial dysfunction, the expression of both chaperones is significantly increased, a process that aids in the repair of damaged proteins and the restoration of mitochondrial function. The results demonstrated that, compared with the control group, the fluorescent expression of *hsp-6*::GFP and *hsp-60*::GFP in transgenic nematodes fed LS232 was considerably higher. Of particular note is the heightened expression of GFP in the intestines and tails of nematodes fed LS232 *hsp-6* (Figs. 5E and F). These results indicate that LS232 maintains mitochondrial homeostasis in intestinal cells by enhancing UPR^{mt} to prolong the life span of its host.

Table 2 Effect of different bacteria on the lifespan of *atfs-1(gk3094)* and *xbp-1(zc12)* mutant

Strain	Group	N	Mean lifespan (days)	Maximun lifespans	Genetic requirement
atfs-1(gk3094)	OP50	75	16.16 ± 0.28	23	NO
	LS232	72	16.50 ± 0.50	23	
xbp-1(zc12)	OP50	70	9.00 ± 1.00	18	YES
	LS232	69	10.50 ± 0.50	19	

Three replicates were carried out. Values with different letters in column are significantly different ($P < 0.05$). Significant differences were analyzed by log-rank (Mantel−Cox) tests. Genetic requirement was defined as "yes" at $P < 0.05$ or "no" at $P > 0.05$; therefore, the specific values were no longer displayed.

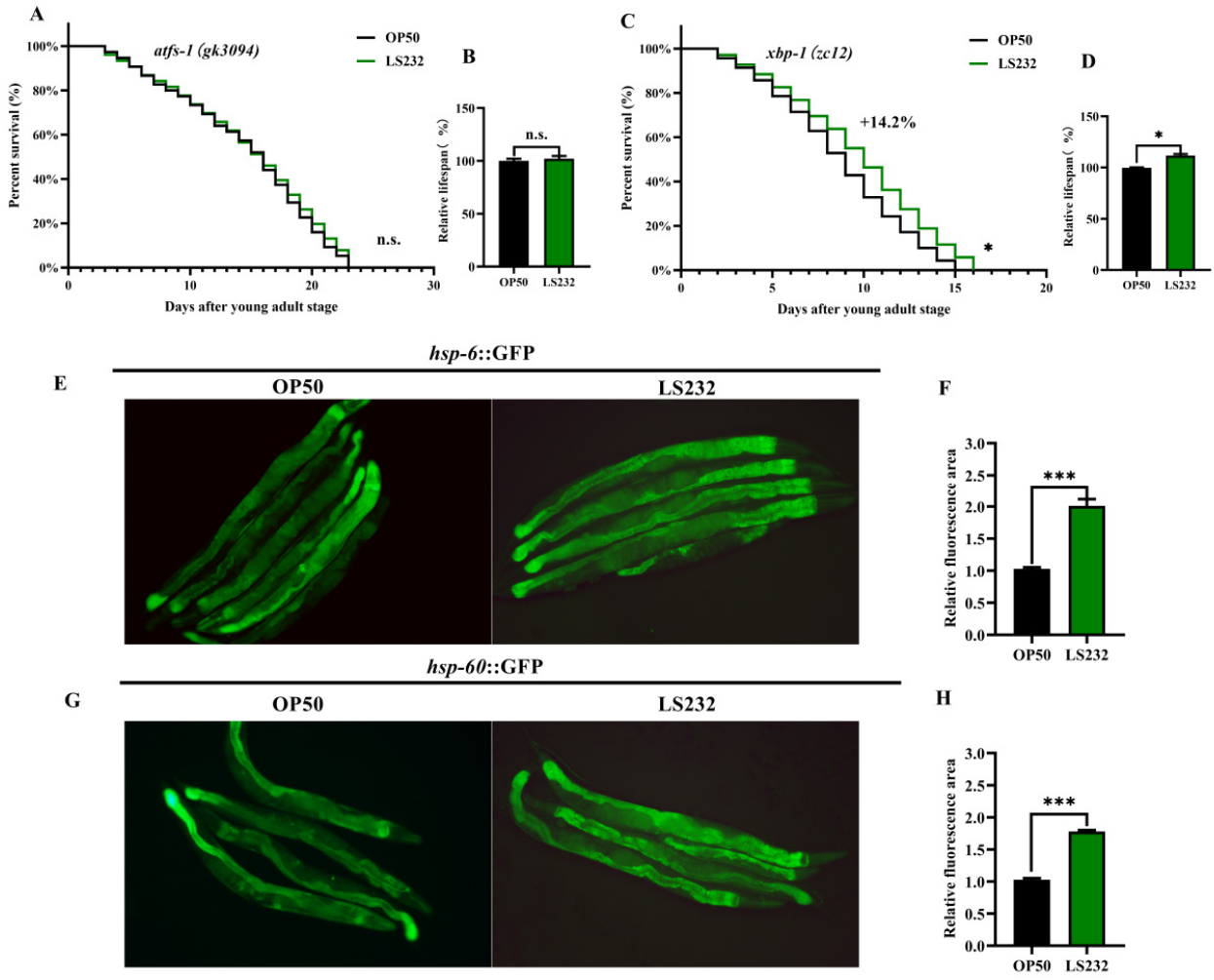

Fig. 5 LS232-induced longevity requires UPR^{mt}. Fig. A shows that LS232 feeding does not extend the lifespan of *atfs-1(gk3094)* mutants. As shown in Fig. B, the lifespan of the nematodes was not extended compared to the control group. Fig. C shows that LS232 feeding extended the lifespan of *xbp-1(zc12)* mutants. Fig. D shows the relative lifespan increase of the worms, indicating that *xbp-1* is a valid mutation. Fig. E shows the fluorescence of the nematode UPR^{mt} representative gene *hsp-60*, detected by GFP. Representative microscopic photographs of *hsp-6*::GFP are shown. Fig. F employs ImageJ software to quantify the fluorescence intensity of *hsp-6*::GFP. The *hsp-6*::GFP nematode grown on LS232 shows an enhanced ability to respond to stress in the intestine and tail. Fig. G demonstrates the fluorescence of *hsp-60*, a representative gene of the UPR^{mt} in the nematode. Fig. H utilizes ImageJ software to quantify the fluorescence intensity of *hsp-60*::GFP. The significance of the results is indicated by the following marking system: (∗) $P < 0.05$, (∗∗) $P < 0.01$, (∗∗∗) $P < 0.001$. It should be noted that all statistical analyses were conducted on the mean ± standard error of three independent experiments.

3.6 LS232 localizes to the nucleus by activating the transcription factor ATFS-1

The results demonstrate that ATFS-1, a transcription factor, modulates mitochondrial oxidative stress and lifespan in *C. elegans*, thereby highlighting its involvement in the activation of UPR^{mt}. Under standard conditions, the coexistence of NLS and MLS within a single transcription activator enables the cell to monitor the overall mitochondrial import efficiency and ascertain the extent of mitochondrial dysfunction. In the event of normal mitochondrial function, ATFS-1, which is continuously synthesized, enters the mitochondria and undergoes degradation. Conversely, as mitochondrial dysfunction escalates (corresponding to cellular senescence), the efficiency of mitochondrial import diminishes, resulting in the transport of ATFS-1 to the nucleus and the subsequent activation of the UPR^{mt} response. Consequently,

mitochondrial homeostasis is preserved through the stress-dependent allocation of transcriptional activators between an inactive state in the mitochondria and an active state in the nucleus[10-11].

Therefore, we determined the effect of nuclear localization of ATFS-1 in nematodes by continuously ingesting LS232 (Fig. 6A). We observed that long-term feeding of LS232 resulted in stronger expression of the ATFS-1::GFP fluorescent protein in the nematode cells than in OP50. Among them, the fluorescent ratio of ATFS-1::GFP in LS232 increased to 55.6% compared with the control (Fig. 6B). These results indicate that ATFS-1 mainly plays an activating role in the gut of *C. elegans*, completes the relevant signal transduction, and mediates the longevity-promoting effect of LS232 in nematodes. These results further support the central role of the transcription factor ATFS-1 in the activation of the UPR^{mt} and the regulation of longevity mediated by LS232.

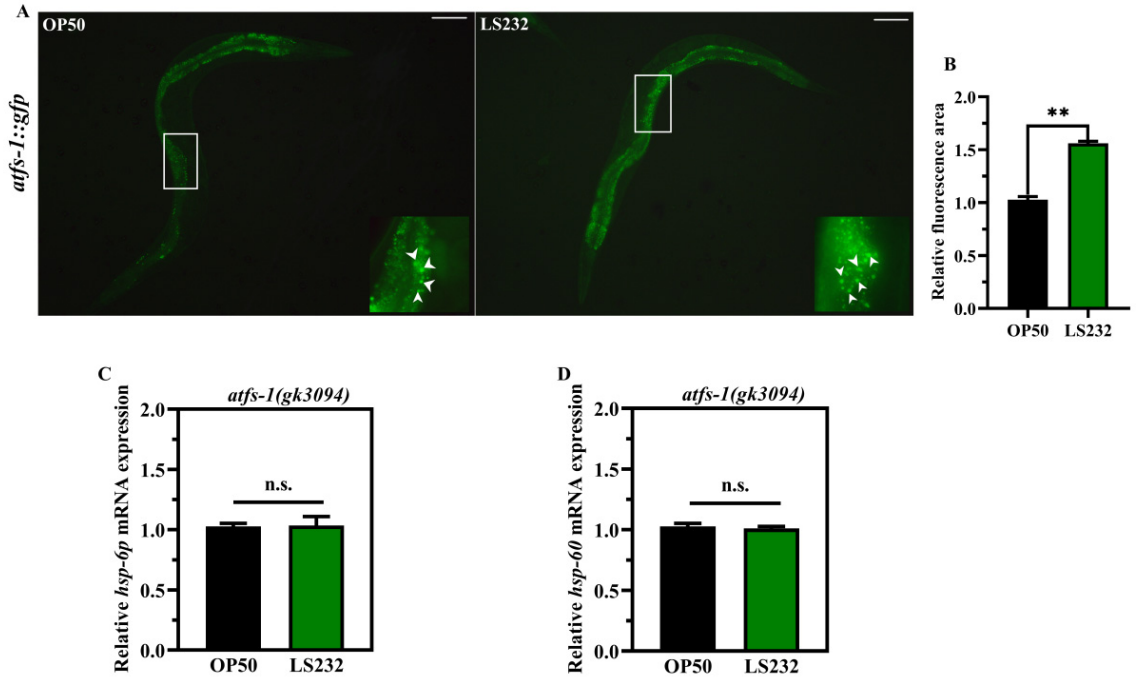

Fig. 6 Feeding LS232 activates the nuclear translocation of the transcription factor ATFS-1 to the intestine. Fig. A shows representative fluorescence microscopy images of ATFS-1::GFP expression in transgenic expressing nematodes after feeding LS232 or OP50 by fluorescence assay (5 days of continuous feeding). The arrow in the right panel indicates the activation process of the nucleus. ATFS-1::GFP aggregates in the intestinal nucleus and emits bright fluorescence. The scale bar is 600μm. As illustrated in Fig. B, the fluorescent intensity of the nuclei ratio of ATFS-1 transferred to the nematode intestine during the feeding of LS232 and OP50 was calculated. The results are shown by SEM of three independent experiments with 30 nematodes per group. In addition, the relative mRNA expression levels of downstream genes (*hsp-60* and *hsp-6*) of the mutant *atfs-1 (gk3094)* after OP50 and LS232 ingestion are shown in Figs. C and D. The statistical significance of the differences between the control and experimental groups is indicated in the figures as follows: (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$. It is imperative to note that all statistical analyses were conducted on the mean ± standard error of three independent experiments.

3.7 Validation of downstream genes of the transcription factor ATFS-1 mutant

ATFS-1, a pivotal transcription factor of the mitochondrial unfolded protein response (UPR^{mt}), plays a critical role in conditions of mitochondrial stress. A comprehensive literature review reveals that ATFS-1 functions as the regulatory hub of UPR^{mt} and is activated dependently[40-41]. To ascertain the role of ATFS-1 as a pivotal upstream regulator of UPR^{mt}, we conducted a specific mRNA detection of downstream genes (*hsp-60* and *hsp-6*) of the mutant *atfs-1 (gk3094)* (Figs. 6C-D). The results of this study demonstrated that

the deletion of the *afts-1* gene led to the absence of transcripts for the *hsp-60* and *hsp-6* genes. This finding indicates that *afts-1* plays a critical role in the intracellular expression of *hsp-60* and *hsp-6* as an upstream gene for their expression.

3.8 The haf-1 gene affects UPR^{mt} signaling by regulating the mitochondrial import efficiency of ATFS-1

The *haf-1* gene, which encodes an ATP-binding cassette (ABC) transporter localized to the inner mitochondrial membrane[42], plays a pivotal role in the unfolded protein response (UPR) in mitochondria (mt). When mitochondrial stress, such as protein misfolding, the *haf-1* gene triggers the UPR^{mt} stress response by facilitating the transport of mitochondrial protein degradation fragments to the cytoplasm. To verify this, the lifespan of the *haf-1(ok705)* mutant was examined. The results demonstrated that the lifespan of the *haf-1(ok705)* mutant remained unchanged after LS232 intake (Figs. 7A and B; Table 3), suggesting that the *haf-1* gene was activated. Furthermore, we conducted downstream gene mRNA detection of *haf-1(ok705)*. The transcript results indicated that the *haf-1* gene is located upstream of the transcription factor ATFS-1 and plays a regulatory role. It is imperative to maintain mitochondrial protein homeostasis (Figs. 7C and D). *Haf-1* facilitates the translocation of ATFS-1 from mitochondria to the nucleus by transporting mitochondrial stress signals (protein fragments) to the cytoplasm. Next, we wanted to determine whether the *haf-1* gene is necessary for the activation of the UPR^{mt}. To test this hypothesis, we performed RNAi interference on the *hsp-6*::GFP nematode. To ensure that the gene was effectively silenced, we measured the relative expression of the *haf-1* gene by RT-qPCR to evaluate the efficiency of the RNAi interference, as shown in Supplementary Figure 1. The results showed that the fluorescent expression of *hsp-6*::GFP, which was initially higher than that of the control group after LS232 feeding, disappeared (Figs. 7G and H). This suggests that knock-down the haf-1 gene may prevent the transcription factor ATFS-1 from entering the cell nucleus for expression, thereby blocking the activation of mitochondrial UPR^{mt} caused by misfolded proteins and the feedback response to protein stress. This proves that the ABC transporter *haf-1* is an important member of the mitochondrial stress response pathway that induces the expression of mitochondrial chaperone genes.

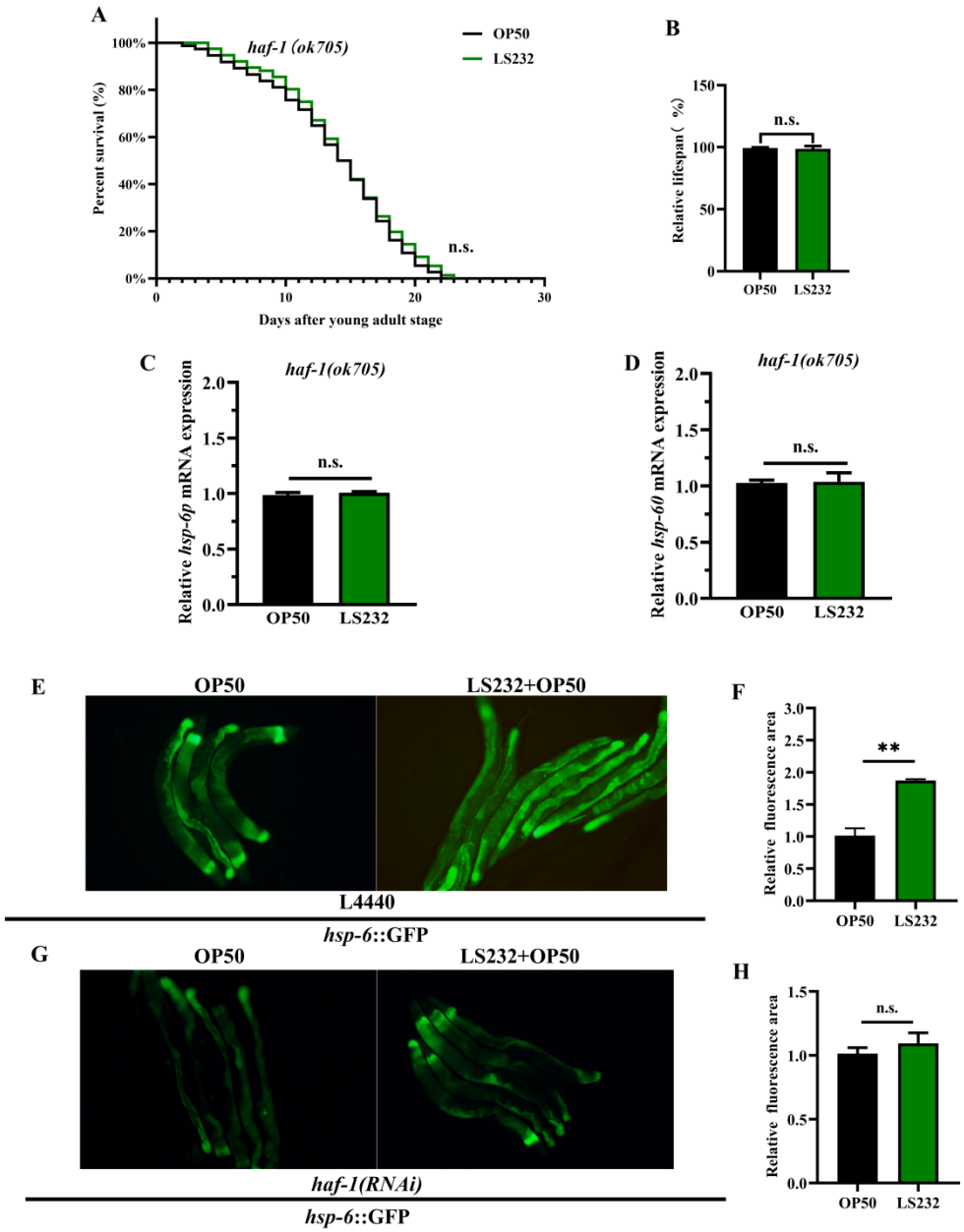

Fig. 7 The *haf-1* gene is crucial for the activation of the UPR^{mt}. As illustrated in Fig. A, LS232 feeding does not extend in the lifespan of *haf-1(ok705)* mutants. Fig. B shows that the lifespan of the nematodes did not increase compared to the control. Figs. C and D illustrate the relative mRNA expression levels of downstream genes (*hsp-60* and *hsp-6*) of the *haf-1(ok705)* mutant. As shown in Fig. E, fluorescent expression of *hsp-6*::GFP was observed after using the empty vector L4440. Fig. F presents the fluorescent area ratio on the left, and it is noteworthy that feeding an empty vector may result in an increase in oxidative stress response. Fig. G presents the fluorescent expression images of *hsp-6*::GFP after feeding OP50 and LS232+OP50 using RNAi. Fig. H displays the fluorescent area ratio on the left, and the fluorescent area of the *haf-1* RNAi nematode is nearly equivalent to that of the control group. The results are expressed as the standard error of the mean (SEM) of three independent experiments, with 30 nematodes per group. The figure is marked as (∗) $P < 0.05$, (∗∗) $P < 0.01$, and (∗∗∗) $P < 0.001$.

Table 3 Effect of different bacteria on the lifespan of *haf-1(ok705)* mutant

Strain	Group	N	Mean lifespan (days)	Maximun lifespans	Genetic requirement
haf-1(ok705)	OP50	74	14.50 ± 0.28	22	NO
	LS232	76	14.50 ± 0.50	23	

Three replicates were carried out. Values with different letters in column are significantly different ($P < 0.05$). Significant differences were analyzed by log-rank (Mantel−Cox) tests. Genetic requirement was defined as "yes" at $P < 0.05$ or "no" at $P > 0.05$; therefore, the specific values were no longer displayed.

4. Discussion

Aging of the body is driven by multiple intertwined mechanisms, including the accumulation of mitochondrial DNA mutations, defects in protein homeostasis leading to instability of respiratory chain complexes, reduced organelle turnover; and changes in mitochondrial dynamics. These changes lead to a decrease in the contribution of mitochondria to cellular energy metabolism, an increase in harmful products. They may cause abnormal mitochondrial membrane permeability, thereby exacerbating inflammation and cell death[43-44]. The robustness of mitochondrial function is essential for health. Its decline is considered to be one of the key drivers of aging. Cells have multiple mechanisms to assess mitochondrial function and activate the UPR^{mt} transcriptional response when mitochondrial integrity and function are impaired. When protein misfolding or dysfunction occurs within mitochondria, the UPR^{mt} senses this stress and activates specific expression programs to restore mitochondrial protein homeostasis by elevating the levels of mitochondrial chaperones and proteases. In recent years, several studies have demonstrated that the role of UPR^{mt} in senescence goes beyond the response to mitochondrial stress. Moderate activation of UPR^{mt} induces an “adaptive stress response” that enhances cellular tolerance to oxidative damage and metabolic disruption, thereby slowing down the aging process in a variety of model organisms[45-46].

Probiotics are defined as “live microorganisms that, when administered in appropriate doses, are capable of producing beneficial health effects on the host[47]”. Probiotics have received widespread attention in recent years as an important factor in regulating host health. They not only have a significant effect on maintaining the intestinal microecological balance and enhancing immune function but also are believed to affect the metabolic state of the host. For instance, in the human gastrointestinal tract, *Lactobacillus plantarum* and other probiotics have been shown to establish and alter the composition and metabolism of the intestinal microbiota, reducing intestinal inflammation and other properties. There have been relatively few studies on the potential of probiotics to improve mitochondrial function and slow aging in the host. First, we systematically evaluated the effect of LS232 on the lifespan and mitochondrial function of *C. elegans* under oxidative stress after ingestion as a harmless food and revealed its mechanism of action. We have confirmed that ingesting LS232 significantly extends the lifespan of *C. elegans* under different environmental conditions while enhancing the nematode's ability to resist oxidative stress. Compared with the control group fed OP50, nematodes fed LS232 had a higher survival rate when exposed to oxidative stress (juglone or UV), indicating that LS232 improves *C. elegans*' tolerance to oxidative stress. This protective effect is consistent with an overall improvement in the function and quality of the nematode cells and mitochondria. Furthermore, the enhanced redox homeostasis capacity of LS232 was observed to result in the restoration of stress resistance in aging nematodes, thereby improving in their cellular and biological characteristics. The study found that LS232 increased the muscle mitochondrial content of nematodes. The intestinal muscle changes of the *ges-1*::GFP (mit) transgenic nematodes demonstrated that, in comparison with the control group, the intestinal muscle and intestinal fiber tissue of the nematode exhibited enhanced orderliness, augmented integrity of the mitochondrial network structure, and diminished number of

mitochondrial fragments. These observations suggest that LS232 may promote the biosynthesis of mitochondria or decelerate age-related mitochondrial degradation. Subsequent analysis of the respiratory activity of the cells revealed that the respiration rate of the nematodes treated with LS232 was marginally higher than that of the control group and remained at a consistent level over time. This effect does not seem to be affected by cellular senescence. Ca^{2+} is essential for optimal mitochondrial function; however, its overabundance can cause damage and eventually lead to cell death. Our observations indicate that the intake of LS232 maintains a stable Ca^{2+} dynamic balance. Finally, we sought to concentrate on the dynamic changes of mitochondrial membrane potential, and we combined the detection of membrane potential with the period of cell aging. The results demonstrated that LS232 intake enhanced red fluorescence production. Collectively, these findings suggest that mitochondrial function is impaired during the aging process, and that LS232 intake can potentially repair this damage. This finding further supports the hypothesis that treatment with LS232 may yield highly effective mitochondria, thereby reducing oxidative stress-induced molecular and cellular damage and promoting a healthy lifespan at the cellular and organismal levels. Then, we conducted an mRNA-based investigation to further explore the potential mechanisms underlying these observations. Our findings indicate that LS232 can activate mitochondrial UPR^{mt}, consequently stimulating mitochondrial protective mechanisms at the molecular level. Treatment with LS232 resulted in a substantial upregulation of UPR^{mt} marker genes *hsp-6* and *hsp-60*, and there was a notable enhancement in the activity of the mitochondrial protective transcription factor ATFS-1. Next, we employed these findings to hypothesize and direct alterations in genes and pathways that contribute to these advantageous effects. The results of the lifespan assays using the UPR^{mt} mutant *atfs-1(gk3094)* and the UPR^{ER} mutant *xbp-1(zc12)* demonstrated that ATFS-1 plays a pivotal role in the longevity and stress resistance effects mediated by LS232. Furthermore, the fluorescent expression results of the mitochondrial chaperone reporter proteins *hsp-6*::GFP and *hsp-60*::GFP indicate that the ingestion of LS232 activates the UPR^{mt} response. Subsequently, we verified the downstream genes of *atfs-1(gk3094)* mRNA and found that *hsp-60* and *hsp-6* transcripts were not expressed, which also confirmed the key role of the transcription factor ATFS-1 in the UPR^{mt}. We determined the effect of ATFS-1 nuclear localization in nematodes by continuously feeding LS232. We observed that after long-term feeding with LS232, the fluorescence of ATFS-1::GFP was expressed more strongly in nematode cells than in OP50. These data suggest that ATFS-1 mainly plays an activating role in the gut of *C. elegans* to complete the relevant signal transduction. Similarly, the upstream regulatory factor *haf-1* has also been reported in the literature to be critical in this process. *Haf-1* encodes an ABC transporter on the inner mitochondrial membrane, which plays a role in UPR^{mt} signaling[48-49]. In the future, our experimental findings revealed that in the *haf-1(ok705)* mutant, the effect of LS232 on nematode lifespan was significantly diminished. Concurrently, the upregulation of UPR^{mt} gene expression (e.g., the enhanced fluorescence of the *hsp-6*::GFP reporter gene) that LS232 intake should induce was no longer observed in the *haf-1* gene knockdown. This finding suggests that the presence of the *haf-1* gene is indispensable for LS232 to induce UPR^{mt}. The *haf-1* gene may facilitate the translocation of the

transcription factor ATFS-1 from the cytoplasm to the nucleus by exporting mitochondrial stress signals (e.g., unfolded protein fragments) to the cytoplasm[50]. Mechanistically, our data suggest that LS232 may induce mild adaptive remodeling of mitochondria, which reduces the import efficiency of ATFS-1 into mitochondria. Under this "import gating," a portion of ATFS-1 accumulates in the nucleus, where it upregulates UPR^{mt} target genes (*hsp-6* and *hsp-60*). In addition, LS232 may enhance overall matrix protein metabolism, leading to increased production of mitochondrial peptides exported by the inner membrane ABC transporter *haf-1*. This peptide export acts as a retrograde signal and, together with impaired ATFS-1 import, synergistically amplifies the UPR^{mt} transcriptional program. These mechanisms provide a clear explanation for the coordinated induction of mitochondrial chaperones and proteases observed after LS232 feeding. Furthermore, another possible reason for LS232 activation of the HAF-1/ATFS-1 axis is that metabolites produced by the bacterium may regulate mitochondrial function and protein homeostasis, which requires further investigation.

In summary, our study has shown that LS232 can improve mitochondrial function and enhance the ability of nematodes to resist oxidative stress by activating the HAF-1/ATFS-1-mediated UPR^{mt} pathway, thereby extending their lifespan. In addition, it is worth exploring whether LS232 has a cross-species effect. The UPR^{mt} pathway and its core regulatory factors are highly conserved in the animal kingdom, so it is speculated that LS232 or its metabolites may also have a mitochondrial protective effect in mammals[51-52]. For example, ATF5 is a functional homolog of ATFS-1 in mammals, the impact of LS232 on the ATF5-mediated mitochondrial stress response could be verified in a mouse model and the specific metabolites responsible for UPR^{mt} activation could be explored.in the future[53]. Overall, the results that LS232 repairs the function of aging mitochondria by activating the UPR^{mt} mediated by the HAF-1/ATFS-1 axis and enhances their antioxidant stress capacity have been verified in *C. elegans*. This finding provides new perspectives and possibilities for applying probiotics in maintaining mitochondrial health and delaying aging in the host.

5. Conclusion

This study proves that the probiotic LS232 can enhance the antioxidant stress capacity and extend the lifespan of *C. elegans* by activating the HAF-1/ATFS-1-mediated UPR^{mt}. In addition, LS232 can delay nematode aging and repair mitochondrial damage. Genetic experiments further confirmed that LS232 activates the repair effect of the HAF-1/ATFS-1 axis-mediated UPR^{mt} on nematodes mitochondria. This finding not only deepens the understanding of the role of probiotics in host health regulation but also provides a new theoretical basis for the future development of probiotic-based repair strategies to improve mitochondrial function and healthy aging.

Supporting Information

All primers used in RT-qPCR experiments (Supplementary Table 1) and see (Supplemental Figure 1) for RNAi interference experiments.

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Data Availability Statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors (ping-biology@outlook.com).

Conflicts of Interest

The authors declare that there is no conflict of interest.

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