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Effect of Feeding Lactic Acid Bacteria from Agave in *Caenorhabditis elegans* Lifespan, Heat Shock and Acute Oxidative Stress

Vania Lizett Lucas-Hernández [1], Liliana Lugo-Zarate [2,3], Diana Patricia Olivo-Ramírez [3], Estefani Yaquelin Hernández-Cruz [4], José Pedraza-Chaverri [4] and Angélica Saraí Jiménez-Osorio [2,*]

[1] Área Académica de Biología, Universidad Autónoma del Estado de Hidalgo, Pachuca 42184, Mexico; lu402975@uaeh.edu.mx
[2] Área Académica de Enfermería, Universidad Autónoma del Estado de Hidalgo, Pachuca 42160, Mexico; lu279379@uaeh.edu.mx
[3] Área Académica de Nutrición, Universidad Autónoma del Estado de Hidalgo, Pachuca 42160, Mexico; diana_olivo@uaeh.edu.mx
[4] Departamento de Biología, Facultad de Química, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico; estefani.hernandez@quimica.unam.mx (E.Y.H.-C.); pedraza@unam.mx (J.P.-C.)
* Correspondence: angelica_jimenez@uaeh.edu.mx; Tel.: +52-7717172000 (ext. 41526)

Abstract

The food industry has a strong interest in lactic acid bacteria (LAB) because of their probiotic potential and health advantages. LAB have been previously isolated from pulque and agave sap, showing antibacterial action. However, their reaction to stress can limit their survivability, and their biological activities are strain-specific. To ascertain the impact of LAB isolated from pulque and agave sap on lifespan, thermal and oxidative stress, and health span parameters, we fed the nematode *Caenorhabditis elegans* these bacteria. The nematodes fed the *Escherichia coli* OP50 strain were utilized as a control for each experiment. Animals were fed each strain for four days starting from L4 and either (day 5) exposed to oxidative stress caused by high hydrogen peroxide concentrations (8 mM) or acute heat stress (35 °C) for four hours. The strains *Lacticaseibacillus rhamnosus* and *Lactiplantibacillus plantarum* significantly improved lifespan, fertility, movement, and heat shock resistance. *Lacticaseibacillus casei* enhanced the *C. elegans* lifespan, and *Levilactobacillus brevis* only increased its survivability in the heat shock studies. Interestingly, we discovered a harmful impact on animals fed *Pediococcus acidilactici.* This study highlights that, even when strains come from the same plant source, their biological activity might differ significantly.

Keywords: *Caenorhabditis elegans*; lactic acid bacteria; oxidative stress; thermal stress

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1. Introduction

Lactic acid bacteria (LAB) are known for their role in food fermentation due to their excellent probiotic properties [1–3], which contribute to intestinal health by supporting homeostasis and protection against pathogens [4,5]. They have also been reported to exhibit antimicrobial [6–8] and antioxidant activities, enabled by their metabolism and the production of organic acids, bacteriocins, exopolysaccharides, and antioxidants that could control systemic homeostasis through probiotic actions [9–11].

LAB have developed various ways to respond to environmental challenges to survive, including changing their metabolism, producing special molecules, and using complex repair and control systems. It has been recognized that LAB possesses a stress-inducible

system to survive against environmental changes such as heat shock, low pH, limited nutrients, and low oxygen [12]. In order to enhance health actions in the host, food technologists must isolate and employ LAB strains with high resistance to gastrointestinal tract conditions.

Moreover, the oxygen sensitivity of LAB may limit their survival in environments characterized by oxidative stress. The environment of the gastrointestinal tract in the host may stimulate the production and activity of reactive oxygen species (ROS). The imbalance between ROS production and elimination causes oxidative stress, potentially damaging proteins, DNA, and lipids, thus disrupting cellular homeostasis and LAB survival [13,14]. As a result, some probiotic LAB strains can help protect the host's intestine from oxidative damage, which has been shown in animal studies, to provide health benefits [15–17]. This response appears to be dependent upon strain-specific capacities for secondary metabolite production and varied antioxidant responses [18].

On the other hand, the viability of LAB is influenced by temperature fluctuations; thus, they have evolved regulatory mechanisms for survival under cold and heat shock by enhancing the expression of the *cold-shock protein A*, *Clp* protease and chaperone genes [19–21]. In recent animal studies, the oral administration of *Lactobacillus reuteri* mitigated testicular damage induced by heat stress [22]. *Lactobacillus plantarum* (*L. plantarum*) *L19* showed protective effects against heat stress-induced hepatic damage by modulating gut microbiota [23]. Heat stress response has also been studied in *Caenorhabditis elegans* (*C. elegans*), where LAB promotes resistance to heat shock and oxidative stress, as well as increased longevity [24,25]. This is a well-established model organism that offers a reproducible and accessible system for identifying strains with biological activity, making such studies valuable for understanding LAB responses to stress and their potential applications [26,27].

LAB strains isolated from plant sources are particularly intriguing in the hunt for strains with high tolerance to different stressors, perhaps because they have evolved defense mechanisms against environmental stimuli. LAB were previously isolated and identified in Mexican Agave [28] from Hidalgo, Mexico, which is used to produce pulque and agave syrup (aguamiel), traditional fermented beverages with health benefits. According to local producers, the taxonomic identity of this plant is unknown; however, current research confirms that *Agave salmiana* and *Agave americana* are the predominant species used for pulque and sap production in Hidalgo State [29]. Cervántes-Elizarrás et al. isolated *L. plantarum* and *Pediococcus acidilactici* (*P. acidilactici*) from sap and *Levilactobacillus brevis* (*L. brevis*) from pulque, exhibiting antibacterial activity against *Helicobacter pylori* in in vitro studies [28]. In addition, when these strains were inoculated in a consortium in blackberry juice, their fermentation for 16 h increased the production of antioxidant compounds and organic acids [30]; nevertheless, their effects on living organisms regarding potential resistance to oxidative and heat shock stress remain unexplored.

Since LAB are widely used in making functional foods and their health effects can vary by species and strain, it is important to test how well they can handle heat and oxidative stress in living organisms to find the strains that are more resistant. Therefore, this study aims to evaluate how five LAB strains react to oxidative and heat stress and how these strains affect the average and maximum lifespan of *C. elegans*. This approach may serve as a helpful tool for selecting LAB strains for food fermentation based on their survival and significant resistance to environmental stresses.

2. Materials and Methods

2.1. Lactic Acid Bacteria Strains

The LAB strains used were *L. plantarum* and *P. acidilactici* from agave sap and *L. brevis*, *Lacticaseibacillus rhamnosus* (*L. rhamnosus*), and *Lacticaseibacillus casei* (*L. casei*)

from pulque. The aforementioned strains were previously isolated as described by Cervantes-Elizarrarás et al. [28]. Samples of pulque and agave sap were gathered in September 2015 from producers in Hidalgo, México. Each sample was diluted 1:50 in Man Rogosa Sharpe (MRS) broth (BD, Sparks, MD, USA) and incubated at 37 °C for 24 h. The identification of LAB was conducted using a polymerase chain reaction with the 16S rRNA gene sequencing (GeneBank accession number: *L. plantarum*, *KT025937.1*; *P. acidilactici*, NR_042057.1; *L. brevis*, *NR_116238.1*; *L. rhamnosus*, OQ784244.1, and *L. Casei*, NR_041893.1). To activate LAB, 100 µL of frozen stocks were inoculated into 5 mL of sterile MRS broth and incubated at 37 °C for 24 h; this was repeated again to complete two cycles. The culture was subsequently concentrated 1:10 using centrifugation at 8000× *g* for 15 min. After this process, the colony-forming units for each LAB strain were evaluated, yielding an average of 9.44 log 10 CFU/mL.

2.2. Maintenance of Caenorhabditis elegans

The N2 Bristol strain of *C. elegans* and its non-pathogenic food source, *Escherichia coli* (*E. coli*) OP50, were obtained from the Caenorhabditis Genetics Center (CGC, Minneapolis, MN, USA) by Dr. José Pedraza Chaverri of the Universidad Nacional Autónoma de México (UNAM). The project was registered by the Institutional Committee for Care and Use of Laboratory Animals (Registration number: CICUAL-V-I/06/2024). Thermal and oxidative stress tests were performed in Laboratory 1 of the Bioterium at the Universidad Autónoma del Estado de Hidalgo, Mexico, while lifespan tests were carried out in Laboratory 315 of Building F at the Faculty of Chemistry, UNAM.

All experiments were carried out using 35 mm Petri dishes containing 3 mL of Nematode Growth Medium (NGM) following standard procedures [31] and maintained at 20 °C. The OP50 cultures were grown for 24 h at 37 °C in Luria Broth medium, concentrated 1:10 by centrifugation (8000× *g*, 15 min), and 100 µL of concentrated bacteria were added to 35 mm Petri-dish with NGM. For the experiments with LAB, 100 µL of centrifuged LAB strains (9.44 log 10 CFU/mL) were placed in the 35 mm Petri dishes containing 3 mL of NGM. All animals were transferred daily to fresh plates with bacteria to prevent contamination.

2.3. Synchronization of Larval Stage

For all experiments, animals were required at the L4 stage. Synchronization was achieved by timed egg laying and bleaching using a 1:0.5 mixture of sodium hypochlorite and 1N NaOH, following Wilkinson et al. [32] with some modifications. Gravid adults were washed with M9 buffer (3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 1 mL $MgSO_4$ 1M, adjusted to 1 L with H_2O), collected with a pipette tip, and centrifuged (8000× *g*, 45 s). The supernatant was reduced to 500 µL, and 300 µL of bleaching solution was added, mixed for no longer than 2 min, and centrifuged again (8000× *g*, 45 s). The supernatant was discarded, and the washing process with M9 buffer was repeated six times. Eggs were then transferred with a micropipette to fresh OP50 plates and incubated at 20 °C until reaching the L4 stage (48 h), yielding a synchronized population.

2.4. Lifespan Assay

Lifespan and health span assays were performed as described by Yu et al. [33]. Twenty synchronized L4 nematodes were placed on each plate containing OP50 or each LAB strain (two plates for each experiment, two experiments, 80 animals in total). Animals were transferred daily to fresh plates, and deaths were recorded. Nematodes unresponsive to mechanical stimulation with a platinum wire were considered dead; those lost to plate walls or with exposed vulva were censored. Survival curves were generated as described in the statistical analysis.

2.5. Healthspan Assays

2.5.1. Progeny Assay

For each experiment, ten synchronized L4 nematodes were selected and placed on plates containing the different bacterial treatments. Animals were maintained at 20 °C and transferred daily to fresh plates. Progeny counts were recorded daily for each of the 10 nematodes until egg laying ceased.

2.5.2. Locomotion Assay

To evaluate nematode motility, ten animals from each group were transferred to fresh NGM plates. After reaching adulthood (day 2 of exposure), sinusoidal body bends were observed and recorded for 30 s every 48 h. Data were collected until the end of the experiment and analyzed by standardizing against the OP50 control.

2.5.3. Pharyngeal Pumping Assay

Ten synchronized L4 nematodes per treatment were placed on plates with the corresponding bacteria. Every third day, pharyngeal contractions were observed and counted for 60 s for each worm until all nematodes died. Results were reported as the average number of pharyngeal contractions per minute.

2.6. Thermotolerance Assay

Nematodes were maintained at 20 °C. Following Rubio-Tomás et al. [34], twenty L4 larvae per plate (two plates of twenty worms per plate) were transferred to OP50 or each LAB strain for 4 days and transferred daily to fresh plates. On day 5, nematodes were exposed to 35 °C for 4 h, then returned to 20 °C. Survival was monitored daily until all animals died. Death was defined as no response to platinum wire stimulation; animals lost to the plate walls or those that hatched internally were censored. Kaplan–Meier curves were obtained for each strain.

2.7. Oxidative Stress Tolerance Assay

Multiple approaches for producing oxidative stress have been recorded utilizing hydrogen peroxide concentrations between 1 mM and 8 mM. We selected the highest concentration based on the methodology reported by Yu et al. to assess oxidative stress tolerance in an acute regimen to determine the maximal resistance that each strain provides to the nematode [33]. Forty synchronized L4 nematodes per plate (two plates of 20 worms per plate) were fed with OP50 or LAB strains for 4 days. On day five, animals were exposed to hydrogen peroxide at a final plate concentration of 8 mM, without food, until they no longer responded to mechanical stimulation with a platinum wire.

2.8. Statistical Analysis

In all analyses, worms fed with *E. coli* OP50 served as controls. Kaplan–Meier survival analysis was performed, and log-rank (Mantel–Cox) tests were applied to compare survival curves and determine significant differences in median lifespan among LAB strains. One-way ANOVA with Dunnett's test was employed to assess significance in health span experiments, and $p < 0.05$ was considered significant. All experiments were performed in duplicate. We utilized GraphPad Prism v8.0.1. for graphical representation and statistical analysis.

3. Results

3.1. Lifespan of Nematodes

Nematodes fed with *L. rhamnosus* showed the highest increase in median lifespan of 33.3% and reached a maximum of 25 days versus OP50 (median life: 10; maximum life: 15). Animals fed with *L. casei* promoted a 16.6% increase in lifespan, and feeding with *L. plantarum* resulted in a minimum increase in mean lifespan (9%) and a maximum life of 20 days. In contrast, *L. brevis* and *P. acidilactici* showed a decrease in lifespan versus OP50 (Figure 1).

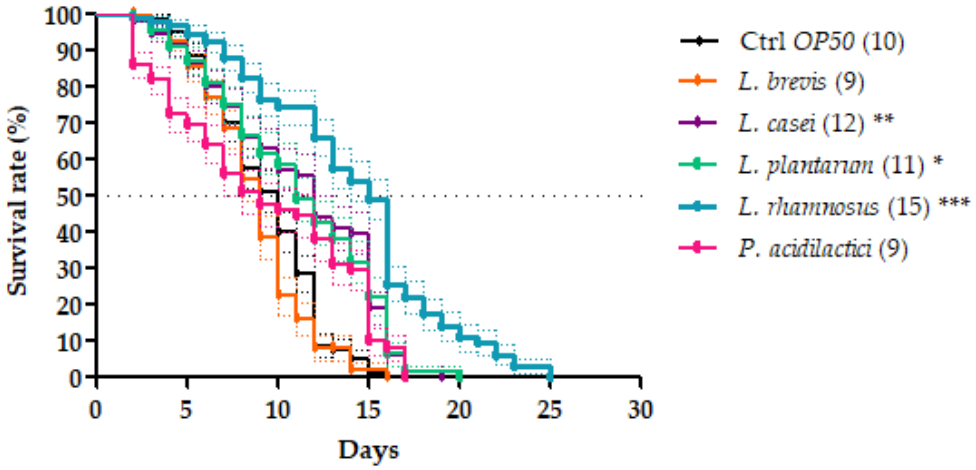


Figure 1. *Caenorhabditis elegans* lifespan at 20 °C fed with OP50 or five different lactic acid bacteria strains (n = 80 animals per strain). Numbers inside the parentheses indicate median survival versus OP50: *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$.

3.2. Healthspan

Determination of total progeny indicated favorable reproductive conditions. *L. rhamnosus* significantly increased progeny number, whereas *P. acidilactici* significantly decreased it (Figure 2a).

In addition, locomotion analysis demonstrated that feeding nematodes *L. rhamnosus* across their lifespan allowed them to maintain motility until the end of their lives (Figure 2b). *L. plantarum* and *L. rhamnosus* enabled the nematodes to maintain oviposition until day 14 (Figure 2b).

Pharyngeal pumps were more active and evident during the first 7 days of life, serving as an indirect indicator that animals were not under fasting conditions. As shown in Figure 2c, most strains maintained pharyngeal contractions until day 9, whereas *L. rhamnosus* sustained them until day 15.

3.3. Thermotolerance

Animals were fed with each tested strain for 4 days and exposed to heat shock (35 °C) for 4 h at day 5. After recovery at 20 °C, median and maximum lifespans were determined. Nematodes fed *L. rhamnosus* showed the highest resistance, with a median lifespan of 15 days (50% higher than the control with *E. coli* OP50, 10 days) and a maximum lifespan of 21 days. *L. brevis* also provided significant protection, increasing the mean lifespan to 12 days (20% higher than the control) and the maximum lifespan of 16 days. *L. plantarum* produced a more modest increase in lifespan versus control (11 days, 10% higher than the control) and a maximum lifespan of 16 days. In contrast, *L. casei* and *P. acidilactici* did not modify mean lifespan compared to the control, although a slight increase in maximum lifespan was observed (16 and 15 days, respectively) (Figure 3).

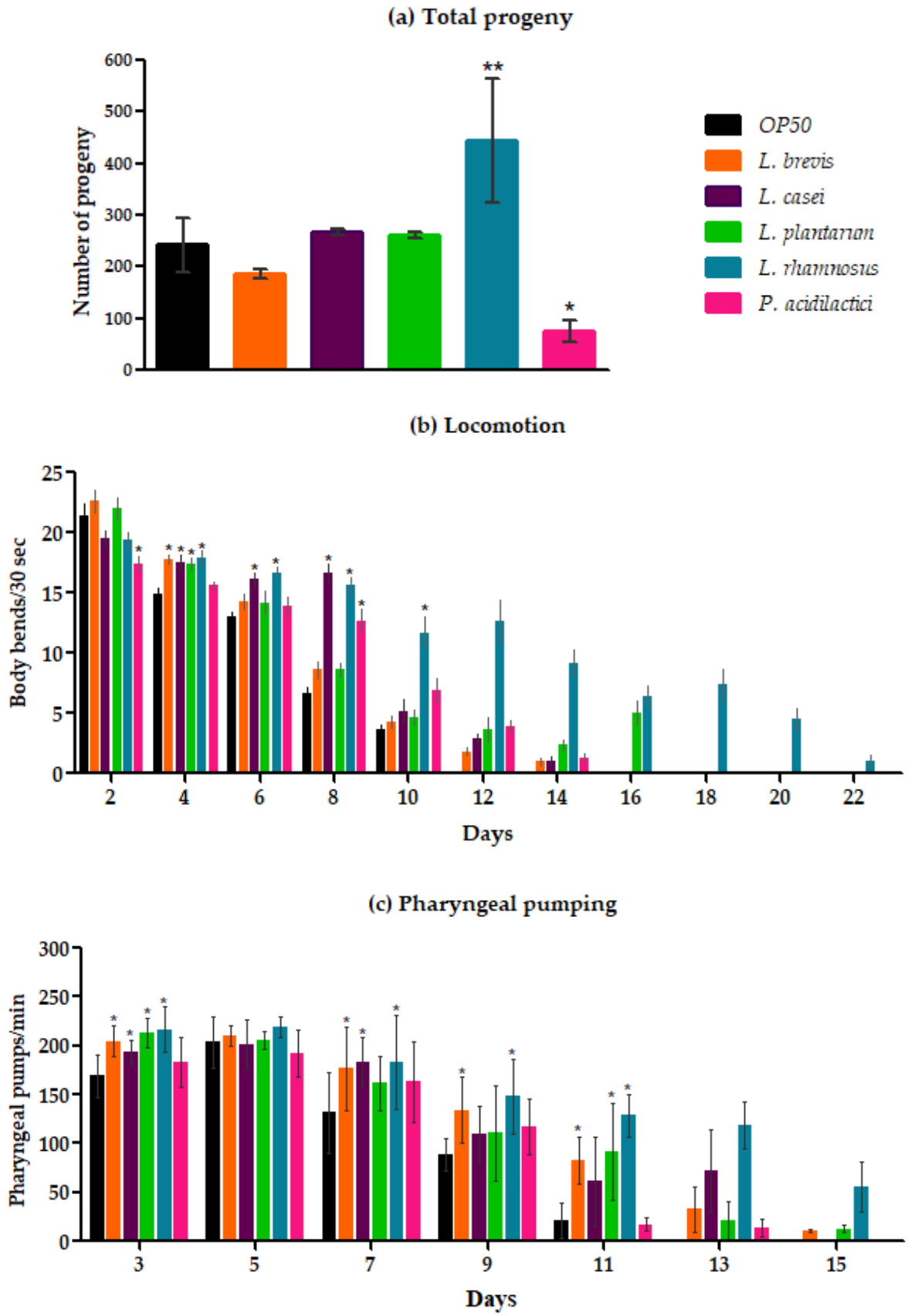


Figure 2. Health span parameters of *Caenorhabditis elegans* fed OP50 and the five LAB tested. (**a**) Total progeny, (**b**) mobility, measured as body bends per 30 s, and (**c**) number of pharyngeal contractions per minute (n = 20 animals per group). Dunnett's test vs OP50 ** $p < 0.01$, and * $p < 0.05$.

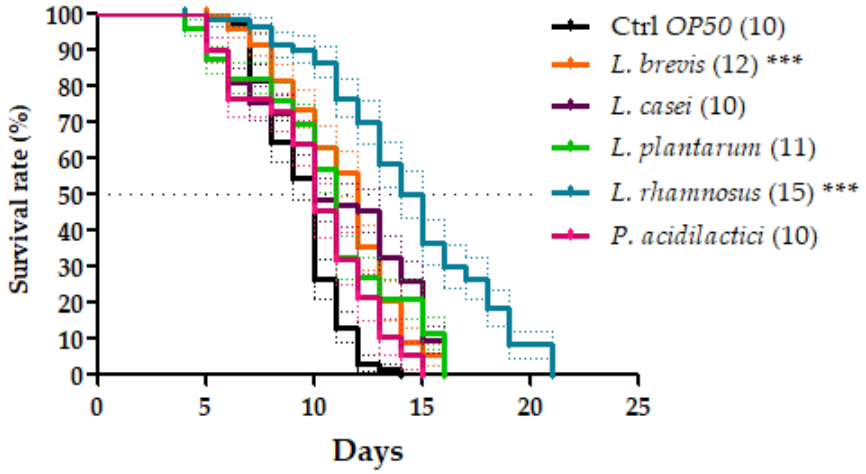


Figure 3. Survival of *Caenorhabditis elegans* fed with OP50 or five different lactic acid bacteria strains (n = 80 animals per strain) after 4 h of heat shock (35 °C), and maintenance of lifespan at 20 °C. Numbers inside the parentheses indicate median survival versus OP50: *** $p < 0.001$.

3.4. Oxidative Stress Tolerance

We analyzed whether pre-exposure for 4 days to different LAB strains could increase the survival capacity of *C. elegans* under oxidative stress induced by hydrogen peroxide (8 mM). We observed that *L. rhamnosus* and *L. plantarum* conferred resistance to oxidative stress by extending maximum survival but not median lifespan. Conversely, *L. brevis* and *P. acidilactici* significantly decreased in median lifespan versus OP50 (Figure 4).

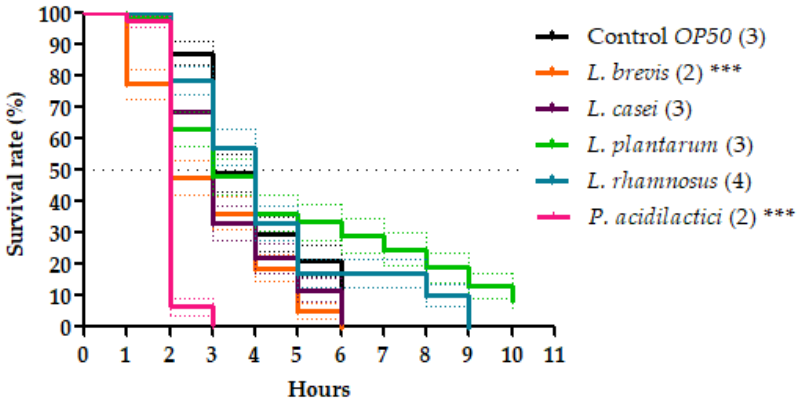


Figure 4. Survival of *Caenorhabditis elegans* exposed to oxidative stress induced by hydrogen peroxide (8 mM) fed OP50 (control) or five different lactic acid bacteria strains (n = 80 animals per strain). Numbers inside the parentheses indicate median survival versus OP50: *** $p < 0.001$.

4. Discussion

This study assessed the effect of feeding the worm *C. elegans* with LAB isolated from agave sap and pulque on its lifespan, health span, and thermal and oxidative stress. Among all the strains assessed, *L. rhamnosus* exhibited the most significant impact on enhancing lifespan, progeny count, motility, pharyngeal pumping, and heat stress tolerance. These findings align with previous reports indicating that LAB can activate conserved pathways of longevity and immunity in *C. elegans* [35–37]. Moreover, LAB have been linked to more general health benefits in *C. elegans*, such as changes in the integrity of the intestinal barrier, immune responses, and microRNA expression [36]. These findings reinforce the concept that probiotic strains can exert anti-aging effects through multiple mechanisms, ranging from improved intestinal health to regulation of cellular stress responses.

4.1. Lifespan Extension

In this study, *L. rhamnosus* increased lifespan by up to 33.3% versus OP50. The lifespan extension of *L. rhamnosus* in *C. elegans* has been previously studied, showing an increased rate ranging from 9.6% to 42.9%. Specifically, the highest increase in lifespan extension was observed in Lcr35, (*Lactobacillus casei rhamnosus* 35), originally isolated from the gut flora of a healthy infant [38], increasing lifespan up to 30.7–42.9% versus OP50, and 225–267% versus *C. elegans* infected with *Candida albicans* [39]. *L. rhamnosus R4*, from fermented Xinjiang cheese, increased the average lifespan and maximum lifespan up to 36.1% [40], whereas *L. rhamnosus* CNCM I-3690 protected worms by increasing their viability by 30% and also increased average worm lifespan by 20% [41]. *L. rhamnosus* KF7 from kefir showed a minimal effect, only increasing lifespan by 9.6% and maximum lifespan for 30 days [42]. *L. casei* LBC80R and *L. rhamnosus* CLR2, isolated from fecal samples, showed no effect in *C. elegans* lifespan extension [43]. We suggest these differences could be related to the LAB source, showing a greater effect in those strains isolated from fermented foods, but the intrinsic mechanisms of this hypothesis should be investigated.

In this report, we found that *L. casei* increased the median lifespan of *C. elegans* versus worms fed with OP50 by 16.6%. Previous reports showed controversial effects in lifespan extension, with an increase from 0.23% to 42%. *C. elegans* fed with *L. casei* 63 from a patient with short bowel syndrome lived 38.5% longer than OP50 [44]. However, *L. casei* LBC80R isolated from fecal samples only showed a 0.23% lifespan increase [43]. *L. casei* IDCC 3451 extended lifespan by 25% and improved motility and reduced amyloid beta accumulation by 42% [45]. In other experiments, the increase in lifespan extension was more evident when *C. elegans* was exposed to pathogens. For example, *L. casei* HY2782 increased lifespan in *C. elegans* treated with *Pseudomonas aeruginosa* PAO1 [46], and PA14 [47]. The results showed that the source of LAB and the pretreatment with pathogens could be important factors in inducing a molecular response to increase survival.

L. plantarum showed a minimum significant effect, increasing median lifespan by 9.9% compared to OP50 in this research. This strain has been widely studied and has the highest percent of lifespan extension in *C. elegans*. *L. plantarum* Pic37 isolated from traditional Italian cheese showed an increase in median lifespan of 30%. This strain also increased the survival capacity under in vitro gastrointestinal conditions up to 98% [48]. The *L. plantarum* CJLP133 from Korean kimchi showed an increase in lifespan of 13.89%, using a heat-killed bacterium, and also showed in vitro inhibition of *Enterococcus faecalis*, *E. coli*, *Staphylococcus aureus*, *Yersinia enterocolitica* and *Salmonella typhimurium* [49]. *L. plantarum* K90 enhanced the maximum lifespan by 50% compared to *E. coli* OP50 [50]. The *L. plantarum PFA2018AU* isolated from carrot (*Daucus carota* L.) showed a median lifespan increase of 86.6% compared to OP50 when animals were fed since hatching and maintained at 16 °C. However, this increase in lifespan occurred at the expense of reduced progeny (60% lower than OP50) [51]. *L. plantarum TWK10* prolongs *C. elegans* lifespan up to 26.1% in liquid culture at 25 °C [52]. Finally, Bai et al. [53] showed that *L. plantarum* SY1 extended lifespan by 25.8% compared to OP50 in L4 using floxuridine to prevent egg hatching. These results may suggest that time of LAB exposition and temperature cause diverse effects in the worm response. Therefore, it is imperative to explore several temperatures of exposition from hatching to adult stages to investigate LAB colonization and response to increase lifespan.

We did not observe significant changes in worms fed with *L. brevis*. In concordance with our study, *L. brevis* SDL1411 isolated from Korean fermented soybean paste, a traditional fermented food, showed no lifespan extension when *C. elegans* were fed this strain, using non-killed and heat-killed LAB [54]. Otherwise, *L. brevis* from Brahva Laboratories increased lifespan in *C. elegans* up to 15.9% versus OP50 in L4 young adult worms, and 14.3% in the L1 stage. The molecular mechanisms investigated showed that *L. brevis* in-

creased lifespan mediated by the increased expression of *daf-16*, *skn-1*, *sod-2*, *hsp-16.2* and *gst-4* [55]. However, the lifespan increased when C. *elegans* were fed *L. brevis* SDL 1411 24 h before being fed *P. aeruginosa* PA14 [54]. This revealed that the time of LAB exposition of 24 h was enough to promote resistance to pathogen invasion.

We observed that *P. acidilactici* reduced *C. elegans* lifespan and did not provide enough protection against oxidative and heat shock stress. This strain possesses the ability to produce lactic acid and reduce the pH, which in turn could be deleterious for *C. elegans* survival. In this sense, Fasseas et al. also reported a reduction in lifespan using killed and not-killed *P. acidilactici*. The exact reason is not clear, but the authors suggest that LAB need to grow and help protect the gut from harmful factors such as pathogen invasion; however, the authors think the lifespan reduction might have occurred because this strain can stop the growth of tumor-like germ cells and prevent egg hatching in *C. elegans* [56].

4.2. Healthspan Parameters

Health span in *C. elegans* was evaluated by diverse parameters such as fertility, locomotion, and pharyngeal pump. The impact of LAB on fertility highlights the complexity of probiotic–host interactions—certain strains promoted reproductive capacity (*L. rhamnosus* and *L. casei*) and others reduced total progeny (*P. acidilactici*). Chelliah et al. [54] reported similar results. The authors found that worms fed 35 LAB strains showed lower fertility than those fed OP50. Further analysis revealed that animals given heat-killed LAB had higher fertility. Fasses et al. showed that *C. elegans* fed *P. acidilactici* had reduced egg hatching. Yu et al. [57] also indicated that active bacteria use resources and energy in the gut of nematodes to sustain survival and reproduction. To elucidate these disparities, it is imperative to specify how each strain employs nutrients and influences energy expenditure to maintain host fertility. This variability suggests that the differences in reproduction may be linked to how each strain adjusts its regulatory processes.

Conversely, analyses of mobility and pharyngeal pumping further substantiate the advantageous role of LAB in maintaining physiological functions during aging. *L. plantarum* TWK10 increased crawling and swimming speed as well as body bending more than OP50 and *L. rhamnosus* GG at day 1 of young adulthood and day 5 of adulthood. Interestingly, in their research, Liao et al. analyzed the effect of *L. plantarum* TWK10 in mice and in a clinical trial. The oral administration of *L. plantarum* TWK10 1×10^{10} CFU/day for two weeks reduced serum lactate levels and enhanced exercise performance and glycogen storage [52]. Also, Cheng et al. reported increased body bends and pharyngeal pumping with *L. rhamnosus GG* and *L. rhamnosus* KF7 [42]. The ability of *L. rhamnosus* to maintain locomotion and pharyngeal activity for longer periods indicates that probiotic supplementation may help preserve neuromuscular and feeding functions, which are critical markers of health span.

4.3. Heat Shock

Thermotolerance assays revealed that the protective effects of LAB against heat stress are strain-dependent, with *L. rhamnosus* emerging as the most effective. This observation is consistent with previous studies reporting that LAB can enhance longevity and stress tolerance in *C. elegans* through activation of protein homeostasis and stress-response pathways [58]. *L. brevis* also increased resistance to thermal stress after 3 h of exposure to 35 °C by 21.2% compared with OP50, which was related to increased expression of the *hsp-16.2* gene. In this report, we also observed increased lifespan in *L. brevis* after 4 h of exposure to 35 °C, which could be related to the expression of heat shock proteins, as observed by Thiruppathi et al. [55].

4.4. Oxidative Stress

Similarly, oxidative stress experiments demonstrated that LAB could induce antioxidant systems, enabling them to neutralize reactive oxygen species, chelate pro-oxidant metal ions, and regulate host stress-response genes. These properties may explain their ability to confer protection against oxidative damage and promote redox balance [58]. However, in this research, the oxidative stress resistance was not significant for *L. rhamnosus* and detrimental to *L. brevis* and *P. acidilactici*. Previous studies found that animals given *L. rhamnosus* Lcr35 did not withstand exposure to 3 mM of hydrogen peroxide, and the researchers believe this imbalance is due to the overproduction of *sod-3* and the lack of catalase or peroxidases to effectively break down hydrogen peroxide [40]. In addition, Kumar et al. [59] observed that *L. brevis* MTCC 1759 increases the expression of *sod-3*. The activation of *sod-3* occurs in a *daf-16*-dependent manner. The observed strain-specific differences suggest that distinct molecular mechanisms are involved and need to be investigated, including the activation of transcription factors such as *daf-16* and *skn-1/nrf2* and the regulation of target genes like *sod-3* and *gst-4*, which were observed with *L. brevis* and *L. casei* [46].

Thiruppathi et al. [55] demonstrated that *L. brevis* increased survival by 45% under oxidative stress induced by 10 mM hydrogen peroxide after 2 h. The reduction in intracellular ROS and the increased expression of *sod-3* and *gst-4* could explain the molecular targets for ROS reduction. *L. plantarum* SY1 also showed increased survival by 18.8% under 2 mM of hydrogen peroxide [53]. We were not able to observe increased lifespan survival in worms exposed to 8 mM of hydrogen peroxide. However, it could be useful to investigate the effect against other oxidant agents like paraquat, colchicine or lower concentrations of hydrogen peroxide.

4.5. Limitations and Future Research

Given the discussion, we recognize that the major limitations of this communication were that we did not evaluate the molecular basis of the strain-specific effects, and oxidative stress exposition was under high levels of one oxidative agent. Future research must be designed to evaluate feeding with LAB from hatching to diverse larval stages, as well to evaluate survival at 16 °C to 25 °C. The elucidation of the molecular basis of these effects, the exploration of synergistic interactions with dietary compounds, and the evaluation of translational relevance in higher organisms will advance the understanding of the potential of probiotics isolated from plant sources for their application in the functional food industry. Despite the acknowledged limitations, the findings point to the ability of specific LAB strains, particularly *L. rhamnosus*, *L. casei* and *L. plantarum*, to enhance longevity in *C. elegans*. The implications extend beyond nematode biology, suggesting possible applications in food preservation, gut microbiota modulation, and health promotion [55].

5. Conclusions

LAB derived from plants can confer different abilities to the host, depending upon the specific strain. In this work, we examined five LAB strains isolated from agave products and noted that *L. rhamnosus*. *L. casei* and *L. plantarum* can extend the lifespan of the nematode *C. elegans*; however, only *L. rhamnosus* and *L. brevis* exhibit tolerance to heat stress. Nematodes fed *L. rhamnosus* improved their health span parameters, while those fed *P. acidilactici* exhibited detrimental effects on the nematode. These findings may be pertinent to the development of functional foods derived from LAB and strain selection related to their biological effect in the host.

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