Testing living probiotics in *C. elegans* using SydLab™One

Nagi Bioscience delivered *in vivo* findings for the living probiotic strain, *Lactobacillus rhamnosus* GG (LGG)

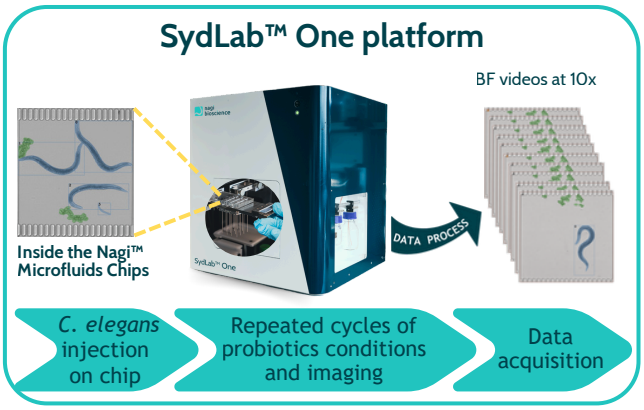
Many studies have focused on the key effects of specific diets and nutrients on longevity, with increasing interest in probiotic supplementation. It has been shown that living probiotics are beneficial to human health by improving intestinal microbial balance, enhancing immune modulation, and/or competing with pathogens (1).

Using the **Sydlab™ One platform,** we explored the effects of the living probiotic *Lactobacillus rhamnosus GG (LGG)* on the physiological functions of *C. elegans*, including lifespan and health biomarkers like mobility and reproduction. Additionally, we deployed **Nagi™ B-Age** to assess the impact of the *LGG* strain on the overall biological age of the nematode. We selected *Lactobacillus rhamnosus GG (LGG),* an FDA-recognized probiotic strain widely used in dietary supplements and fermented foods for its gastrointestinal and immune benefits (2). Emerging evidence suggests *LGG*'s positive effects on urinary infections and vaginal microbiota (3).

Why is *C. elegans* a powerful model to study probiotics and gut health?

C. elegans serves as an exceptional *in vivo* model to explore the interplay between probiotics and host physiology. Its well-characterized gut and nervous systems, together with conserved molecular pathways (p38 MAPK, SKN-1 & CED-1), allow researchers to uncover how microbial metabolites influence healthy aging, metabolism, and overall organismal function. Probiotic interventions in *C. elegans* have demonstrated effects on longevity (4, 5), lipid regulation (6), neurotransmitter balance (7), stress resilience, and even protein homeostasis (8), mechanisms that are directly relevant to human gut-brain and gut-metabolic health. This makes *C. elegans* a highly predictive and efficient system for screening probiotic candidates and elucidating their mechanisms of action before advancing to mammalian or clinical studies.

Dual approach: SydLab™ One platform + Nagi™ B-Age



SydLab™ Analyzer Suite

Real-time data extraction and AI-based analysis

Nagi™ C-age

Mobility (motility & locomotion)
Growth (area)
Reproduction

Nagi™ B-age

Biological age
Vital traits measurements

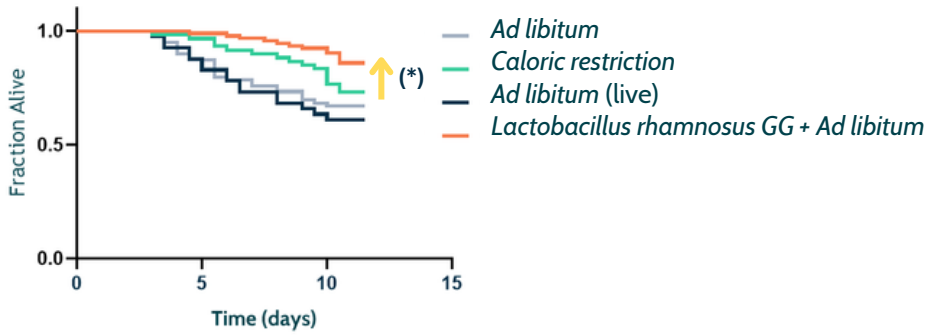
Experimental design:

Wild-type *C. elegans* (N2 strain) were fed live *Lactobacillus rhamnosus GG* (LGG) in place of or alongside the *ad libitum* (f.d. OP50), with various LGG concentrations tested. As a positive control, a restricted f.d. OP50 diet (20% *ad libitum*) was included, given its well-studied benefits on aging and longevity. The effect of live OP50 alone was also evaluated. **Here, we present the comparisons that revealed functional outcomes and distinct biological age shifts.**

Results:

The results below focus on healthspan, **revealing that supplementation with live *Lactobacillus rhamnosus GG (LGG)* increased early-life survival and improved mobility (motility and locomotion).** Improved motility (body-bending frequency) refers to full-body bends per unit of time and assesses neuromuscular coordination, muscle strength, and energy availability. When testing interventions, motility is an early indication of muscle function decline. Locomotion (displacement speed) is the distance the nematode travels in a given time, defining the speed of a movement and assessing the whole-body coordination and motivation to move. **Both mobility parameters are included as part of a holistic study of healthspan to give a comprehensive, clear conclusion.**

Increased early-life survival



Lactobacillus rhamnosus GG combined with ad libitum feeding resulted in increased early-survival compared to the caloric restriction group, suggesting an even greater beneficial effect on longevity.

Figure 1. Survival curves of *C. elegans* populations with different feeding preparations, including supplementation with live *Lactobacillus rhamnosus GG* over 12 days. Survival analyses were performed using the Kaplan-Meier method, and statistical significance between survival curves was calculated using the log-rank test. Significant difference ($p>0.05$) is indicated by an asterisk (*).

Motility

Body bending frequency

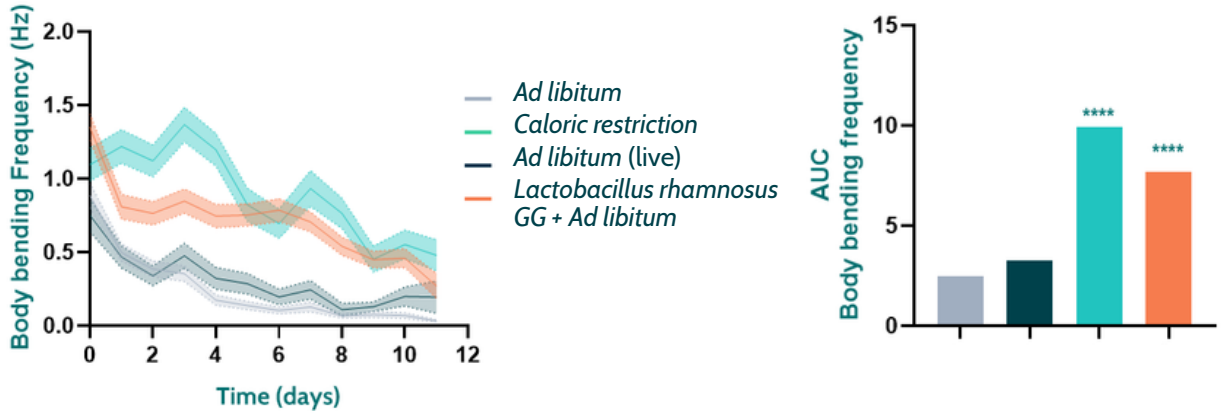


Figure 2. Body bending frequency was measured longitudinally. Statistical analysis was performed using two-way ANOVA to assess overall differences across curves, followed by Bonferroni's multiple comparisons test.
**** $p<0.0001$.

Locomotion

Displacement speed

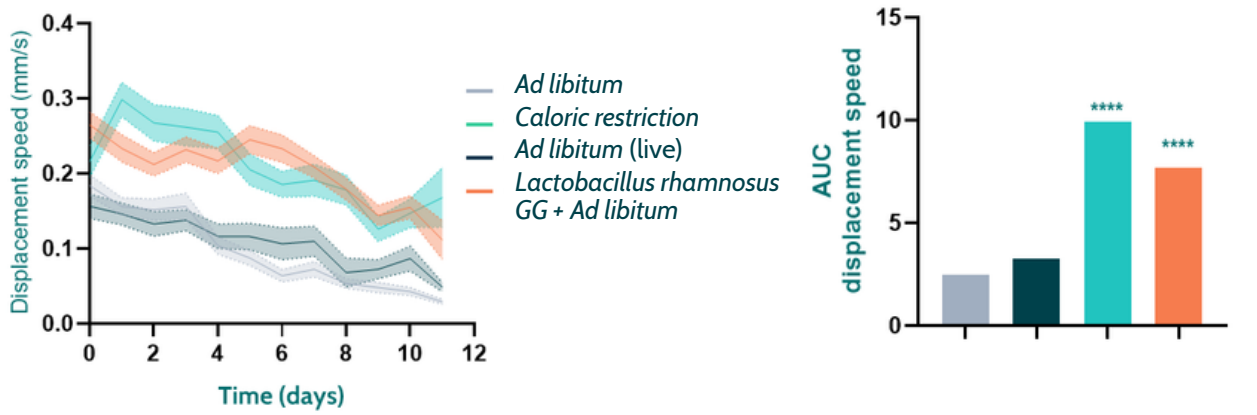


Figure 3. Locomotion was measured longitudinally. Statistical analysis was performed using two-way ANOVA to assess overall differences across curves, followed by Bonferroni's multiple comparisons test. **** p<0.0001.

Growth (size)

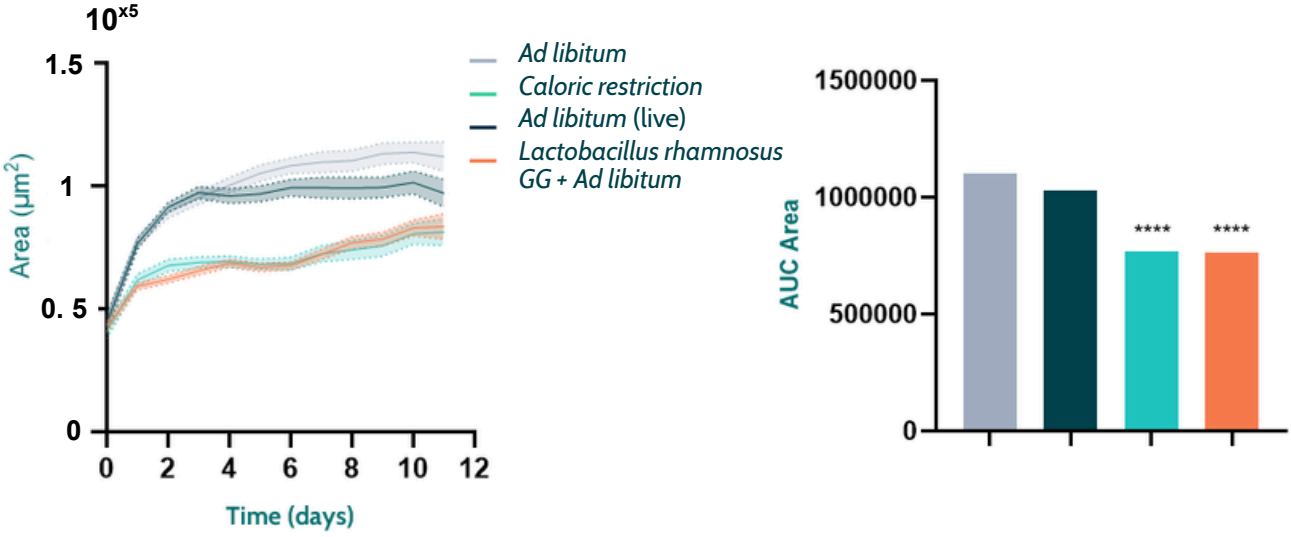


Figure 4. Time-course of worm area (µm²) over 12 days and graphical representation (bar graphs) of *C. elegans*' growth (area under the curves) in different feeding preparations, including supplementation with *live Lactobacillus rhamnosus GG*. p-values were obtained by comparing the negative control (*ad libitum*) to each of the different conditions via 2-way ANOVA to assess overall curve differences, followed by Bonferroni's multiple comparisons test. *** p<0.001; **** p<0.0001

No significant growth is observed. On the contrary, it is presumed that the *C. elegans* population, which is fed with live *Lactobacillus rhamnosus GG,* mimics the phenotype of caloric restriction suggesting metabolic reallocation.

Reproduction

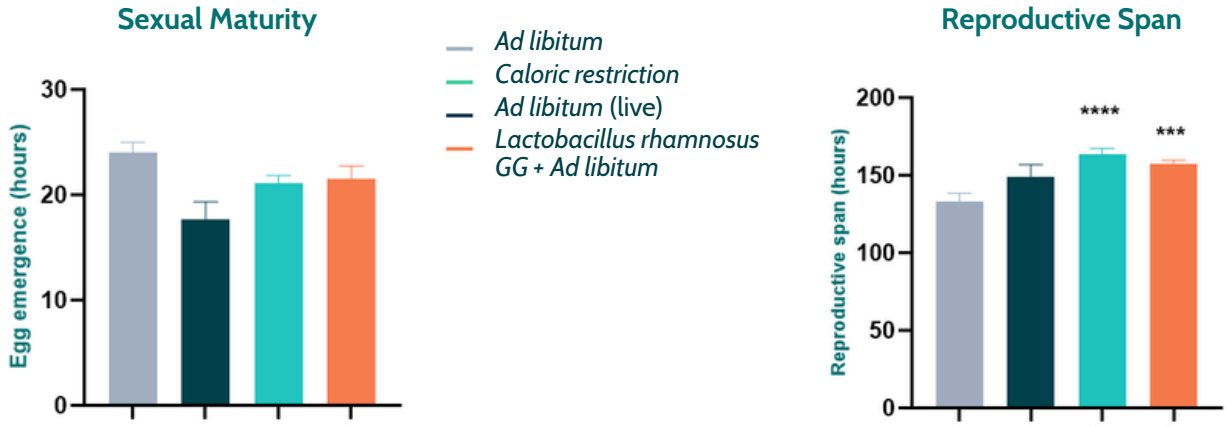


Figure 5. Graphical representation of C. elegans reproductive dynamics under different feeding conditions, including supplementation with live Lactobacillus rhamnosus GG. Reproductive span and timing of sexual maturity were analyzed across groups. The average number of eggs laid per nematode during the reproductive window was also calculated, with no significant differences observed (data not shown). Statistical analysis was conducted using one-way ANOVA comparing the ad libitum control to all other conditions, followed by Bonferroni's multiple comparisons test. ***p < 0.001; ****p < 0.0001

Supplementation with live *Lactobacillus rhamnosus GG* extended the reproductive span of *C. elegans* without increasing total egg output or affecting egg viability. **The fact that the reproductive system stayed functional longer without increasing the total effort (no significant difference in egg emergence or total number of eggs) suggests sustained energy balance and stabilized reproductive performance. These findings support the concept of a trade-off between reproduction and longevity/maintenance, a well-studied theory across organisms, including *C. elegans*.**

Biological age measurement

To further investigate the impact of the probiotic strain *Lactobacillus rhamnosus GG* on healthspan, we used Nagi™ B-Age to quantify the biological age of *C. elegans* and identify trait-specific improvements.

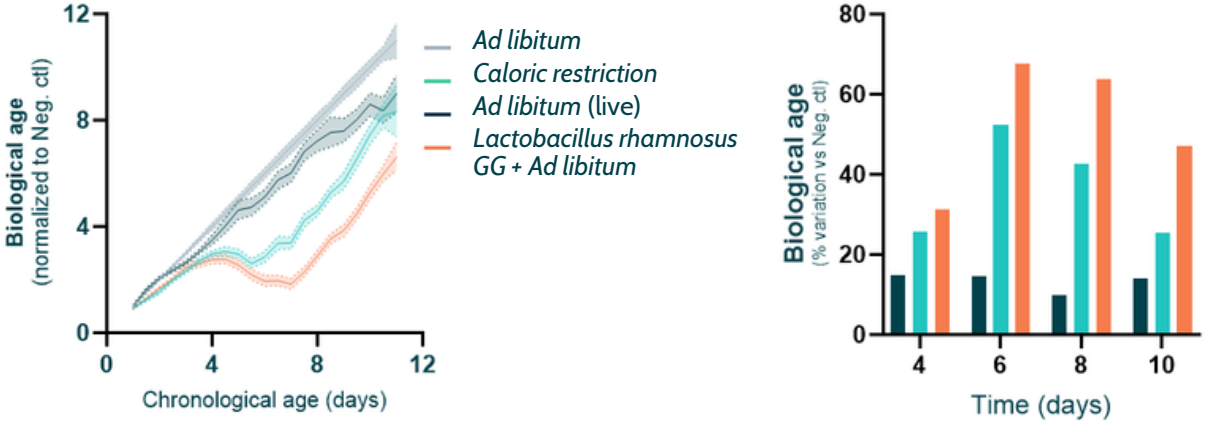


Figure 6: Evaluation of the probiotic strain *Lactobacillus rhamnosus GG* impact on *C. elegans* healthspan using Nagi™ B-Age. Biological age prediction by Nagi™ B-Age, from day 0 to 12, across different feeding conditions. A barplot illustrates the variation in predicted biological age compared to *ad libitum* at different time points. Statistical significance was assessed with two-way ANOVA followed by Bonferroni's multiple comparisons test. No statistical significance was observed.

The **Nagi™ B-Age** prediction was further decomposed into individual vital traits using a multiparametric approach that evaluates over 20 phenotypic biomarkers. These traits represent how the organism allocates energy already by date 8.

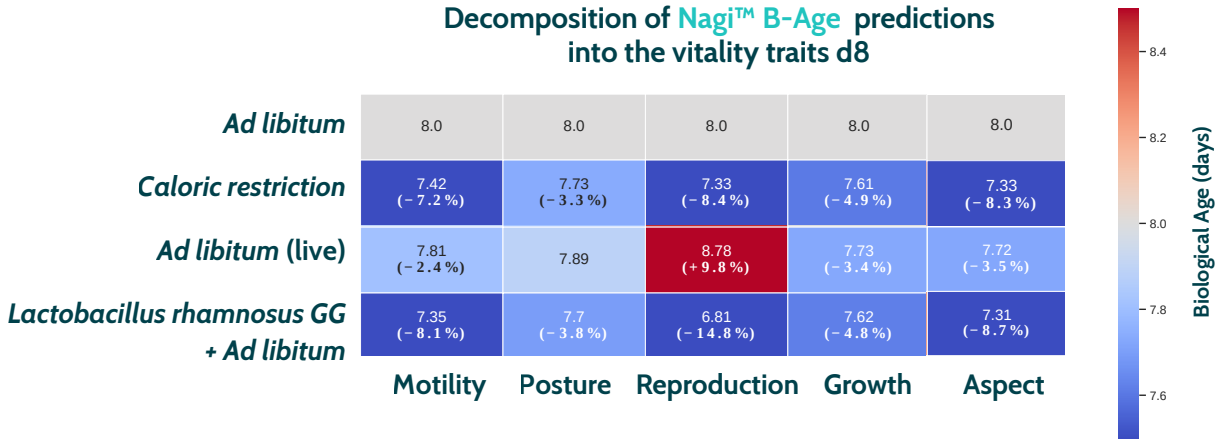


Figure 7: Decomposition of Nagi™ B-Age predictions into the vitality traits at day 8. Significant changes in biological age, relative to *ad libitum*, are expressed as percentage improvements or deteriorations. The overall change in biological age reflects the cumulative effect of changes across all vital traits.

By day 8, the biological age trajectories of the population fed with the live probiotic strain *Lactobacillus rhamnosus GG* were distinct. The detailed analysis of the five vital traits affirms the enhanced physiological maintenance, indicating that supplementation with live *Lactobacillus rhamnosus GG* leads to lower biological scores across multiple parameters (motility, aspect, and reproduction). This highlights the sensitivity and predictive power of Nagi™ B-Age in identifying aging dynamics early in life.

1. Juntao Zhang. *et al.*, *Communications Biology, 1139*. 2022
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Ready to evaluate the impact of your living probiotic strains?