ROMANIAN ACADEMY
School of Advanced Studies of the Romanian Academy
Institute of Biochemistry

PhD THESIS SUMMARY

Genetic and microbial determinants of longevity in *Drosophila melanogaster*

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Table of contents (Thesis *IN EXTENSO*)

I. GENERAL INTRODUCTION TO AGING RESEARCH

Aging research has evolved from understanding why we age to exploring ways to extend lifespan and healthspan. Key theories include Medawar's mutation accumulation and Williams' antagonistic pleiotropy, which explain aging as a consequence of evolutionary trade-offs. The *hallmarks of aging* framework identifies biological processes like genomic instability, mitochondrial dysfunction, and stem cell depletion as targets for intervention.

Aging is driven by interconnected pathways, including oxidative stress, mitochondrial decline, and insulin/IGF signaling (IIS). ROS-induced damage accelerates aging, while reduced IIS activity extends lifespan, as seen in *daf-2* (C. elegans) and *InR* (Drosophila) mutants. Understanding these mechanisms offers potential anti-aging interventions.

Model organisms like *Drosophila, C. elegans,* and *mice* are essential for aging research. *Drosophila* is widely used due to its short lifespan, genetic tools, and 70% gene similarity with humans. It has helped elucidate IIS, TOR, and gut microbiome interactions in aging. Aging-associated microbiome shifts influence immune responses, with *Drosophila* studies showing microbial balance impacts lifespan and health.

By studying aging pathways in model organisms, researchers aim to develop targeted strategies to slow aging and prevent age-related diseases. *Drosophila melanogaster* remains a key system for testing genetic and pharmacological approaches to aging research.

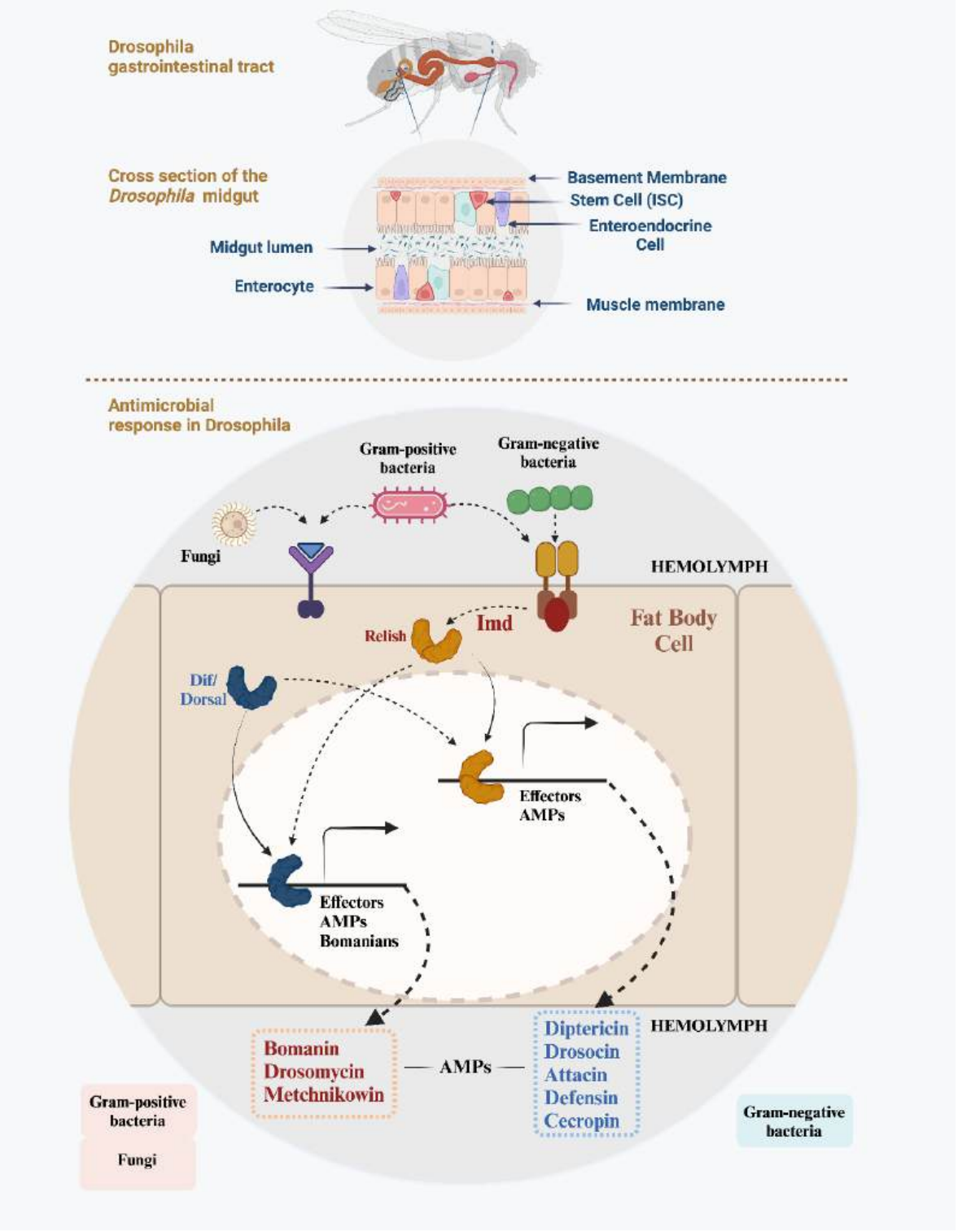


Figure I.1. Gastrointestinal tract and mechanism of AMPs in Drosophila immune response. Adapted from Hanson and Lemaitre, 2020, "New insights on Drosophila antimicrobial peptide function in host defense and beyond," *Current Opinion in Immunology*, 62, 22-30. https://doi.org/10.1016/j.coi.2019.11.008. Created in BioRender. Sarghie, L. (2024) https://BioRender.com/q02f682

II. SYNERGYAGE DATABASE AND ITS ROLE IN AGING RESEARCH

Acknowledgement: Some of the results discussed in this chapter were previously published in: Bunu, G., Toren, D. et al., *Scientific Data* 7, 366 (2020). https://doi.org/10.1038/s41597-020-00710-z

II.1. Introduction to SynergyAge

SynergyAge is a curated database of longevity-associated gene (LAG) interactions, highlighting synergistic and antagonistic effects on lifespan. Predicting interactions among gene mutations is challenging due to epistasis, which SynergyAge addresses by integrating lifespan data from C. elegans, D. melanogaster, and M. musculus. It classifies genetic interactions as additive, dependent, synergistic, or antagonistic, providing insights into aging regulation.

The database features an interactive platform for network-based gene analysis, supporting lifespan study design and predictive modeling. It serves as a key resource for aging research by cataloging lifespan data and facilitating cross-species comparisons.

During my studies, I contributed to SynergyAge curation (Bunu et al. 2020), focusing on D. melanogaster and partly C. elegans data. My role involved collecting and verifying lifespan studies, standardizing gene interactions, and ensuring consistency in gene names and study details. By categorizing interactions, I aimed to enhance accessibility, making SynergyAge a practical tool for understanding genetic influences on aging.

II.2. Methods

To evaluate the impact of combining two genetic mutations, lifespan measurements were taken for four strains: the wild type (WT), two single mutants (G1 and G2), and the double mutant (G1;G2). Each mutant's effect on lifespan was calculated as a percentage difference from the wild type, using the following formulas:

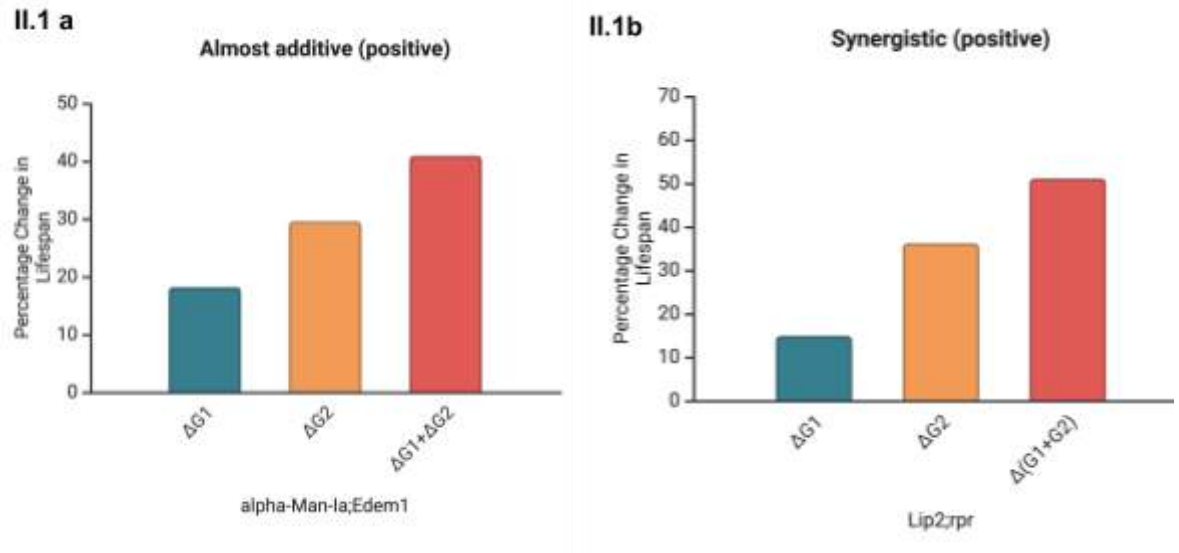
$$\Delta G1 = \frac{(lifespan(G1) - lifespan(WT)) \times 100}{lifespan(WT)}$$

$$\Delta G2 = \frac{(lifespan(G2) - lifespan(WT)) \times 100}{lifespan(WT)}$$

As mentioned in Bunu et al., 2020, for cases where both mutations positively impacted lifespan ($\Delta G1 \times \Delta G2 > 0$), the combined effects were assessed and categorized as follows:

- **Fully synergistic** if $|\Delta(G1,G2)|>|\Delta G1|+|\Delta G2|$|Δ (G1, G2)| > |Δ G1| + |Δ G2|$|\Delta(G1,G2)|>|\Delta G1|+|\Delta G2|$
- **Additive** if $|\Delta(G1,G2)|\approx|\Delta G1|+|\Delta G2|$|Δ(G1, G2)| ≈ |ΔG1| +|ΔG2|$|\Delta(G1,G2)|\approx|\Delta G1|+|\Delta G2|$
- **Almost additive** when $|\Delta(G1,G2)|$|Δ(G1, G2)|$|\Delta(G1,G2)|$ lies between max(|ΔG1|,|ΔG2|)\max(|Δ G1|, |Δ G2|)max($|\Delta G1|,|\Delta G2|$) and $|\Delta G1|+|\Delta G2|$|Δ G1| + |ΔG2|$|\Delta G1|+|\Delta G2|$
- **Dependent (antagonistic)** if $|\Delta(G1,G2)|<\max(|\Delta G1|,|\Delta G2|)$|Δ (G1, G2)| < \max(|ΔG1|, |Δ G2|)$|\Delta(G1,G2)|<\max(|\Delta G1|,|\Delta G2|)$ but greater than min(|ΔG1|,|ΔG2|)\min(|Δ G1|, |Δ G2|)min($|\Delta G1|,|\Delta G2|$)
- **Fully antagonistic** if $|\Delta(G1,G2)|<\min(|\Delta G1|,|\Delta G2|)$|Δ (G1, G2)| < \min(|Δ G1|, |Δ G2|)$|\Delta(G1,G2)|<\min(|\Delta G1|,|\Delta G2|)$

Multiple such epistasis interactions have been curated for SynergyAge. To exemplify, several graphical representations are included below (Figure II.1) to illustrate these interactions among specific *Drosophila* LAG combinations



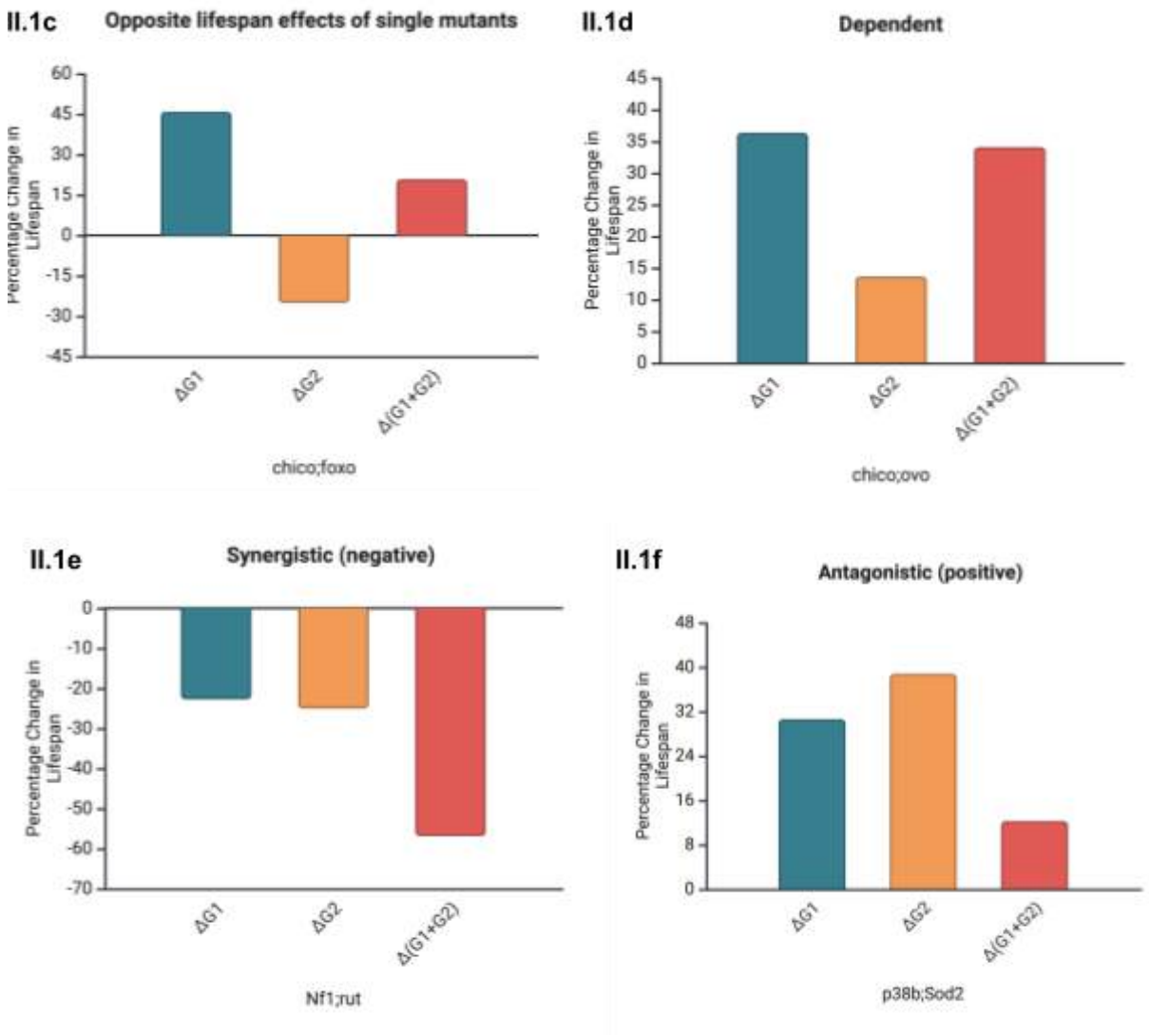


Figure II.1. Graphical Representations of Selected Synergistic and Antagonistic Interactions in *Drosophila* Longevity-Associated Genes (a) **almost additive** (positive), (b) **fully synergistic** (positive), (c) **opposite effects** of single mutants, (d) **dependent** interactions, (e) **negative synergism**, and (f) **positive antagonism.** Created in BioRender. Sarghie, L. (2024) https://BioRender.com/q02f682

II.3. Conclusions

By bringing together data on single and multi-gene mutants, SynergyAge allows us to understand both supportive and opposing effects among longevity-associated genes. Its interactive interface makes it easy to sift through complex data, identify meaningful patterns, and visualize interactions that could shape future research.

For *Drosophila*, where data are still limited, this study highlighted key interaction types, showcasing how SynergyAge can guide research even when data gaps exist. With its

intuitive design and thorough curation, SynergyAge not only provides researchers with immediate insights but also supports the development of more targeted experiments in the quest to uncover the genetic foundations of aging.

III. GENE INTERACTIONS FROM SYNERGYAGE: EXPLORING NOVEL PATHWAYS IN AGING

Some of these results were accepted for publication in Vol. 28, No. 5 (2023) of the *Romanian Biotechnological Letters* journal

III.1. Introduction

Drosophila melanogaster has been crucial in aging research, with nearly 200 longevity-associated genes documented in GenAge (Tacutu et al., 2018). While *C. elegans* has over 1,770 recorded gene interactions affecting lifespan, *Drosophila* remains underexplored, with only 27 known combinations (Bunu et al., 2020). Expanding this research could reveal conserved aging pathways.

This study uses SynergyAge to investigate *Drosophila* gene interactions, focusing on *InR* and *GαO*, orthologs of *daf-2* and *odr-3* in *C. elegans*. It is the first to experimentally assess *GαO*'s role in *Drosophila* aging. Since *InR* regulates the conserved insulin/IGF-1 signaling (IIS) pathway, reducing its activity extends lifespan by enhancing stress resistance (Krishnan et al., 2024). *GαO*, linked to nervous system development and apoptosis, may also influence longevity.

Inspired by *C. elegans* studies showing synergistic lifespan extension, this research tests whether co-manipulation of *InR* and *GαO* in *Drosophila* extends lifespan, offering insights into conserved aging mechanisms.

III.2. Methods

This study integrates multiple data sources to investigate gene interactions affecting longevity in *Drosophila melanogaster*. Protein-protein interactions were obtained from BioGRID, while lifespan-related synergistic gene interactions were identified using the SynergyAge database. Cross-species comparisons between *Caenorhabditis elegans* and *Drosophila* were facilitated using the orthogene package.

For data visualization, Cytoscape (v3.10.1) was employed to map and analyze complex genetic interactions. The experimental *Drosophila* strains, including GαO and InR mutants, were sourced from the Bloomington Drosophila Stock Center, with the

Canton-S strain serving as a control. Flies were cultured on Nutri-Fly™ Bloomington Formulation medium.

To generate double and triple mutants for lifespan experiments, targeted genetic crosses were performed under controlled conditions. This included producing GαO - InR, GαO - eIF, eIF - InR, and GαO - eIF - InR mutants. The crossing strategies, experimental design, and mutant selection were carefully structured and are summarized in Figure III.1.

The lifespan assay was conducted using synchronized groups of *Drosophila melanogaster* (equal numbers of males and females), housed at a density of 27–32 flies per vial. Flies were transferred to fresh food vials every 2–3 days, with mortality recorded throughout their lifespan. Transfers were performed without anesthesia to prevent stress-related mortality, particularly in older flies.

For data analysis, median lifespan was calculated, and Kaplan-Meier survival curves were generated. Group comparisons were performed using the log-rank test, while the Gompertz-Makeham model was applied to assess whether lifespan changes were due to alterations in aging rate or overall healthspan.

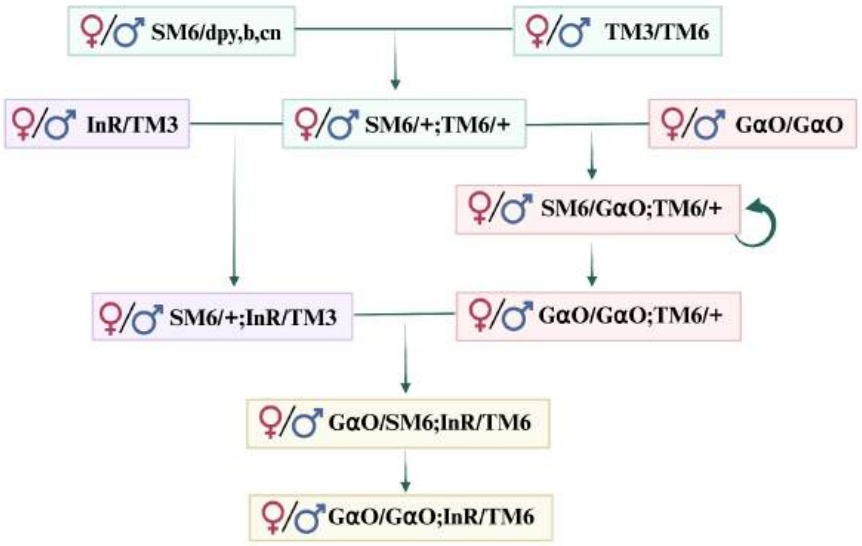


Figure III.1. Crosses to Create the Double Mutant *GαO - InR*.

Straight lines with arrows show crosses between distinct genetic lines, while loops indicate mating within the same line. This figure was previously published in Romanian Biotechnological Letters, currently in press. Created in BioRender. Sarghie, L. (2024) https://BioRender.com/q02f682

III.3. Results and Discussion

This section presents the analysis of networks constructed to investigate longevity-associated genes (LAGs) through identified protein-protein interactions and predicted synergistic relationships. These networks provide a framework for understanding potential lifespan-regulating pathways in *Drosophila melanogaster*, with a focus on critical interactions like those between *InR* and *GalphaO*. By examining network structures, key nodes, and connections, we aim to reveal insights into genetic interactions that influence aging processes.

Additionally, lifespan results for both single and double mutants of *GalphaO* and *InR* are discussed here, highlighting how these specific gene alterations impact lifespan extension. Comparisons between single-gene mutants and the *GalphaO - InR* double mutant reveal differences in longevity outcomes, helping to identify whether combined gene effects lead to additive, synergistic, or independent impacts on lifespan regulation.

One key network (Figure III.2d) focuses on potential LAGs directly connected to GαO and InR, derived from the BioGRID interactome. By examining first-degree interactions, this network highlights primary gene connections that may play a role in lifespan regulation in *Drosophila*.

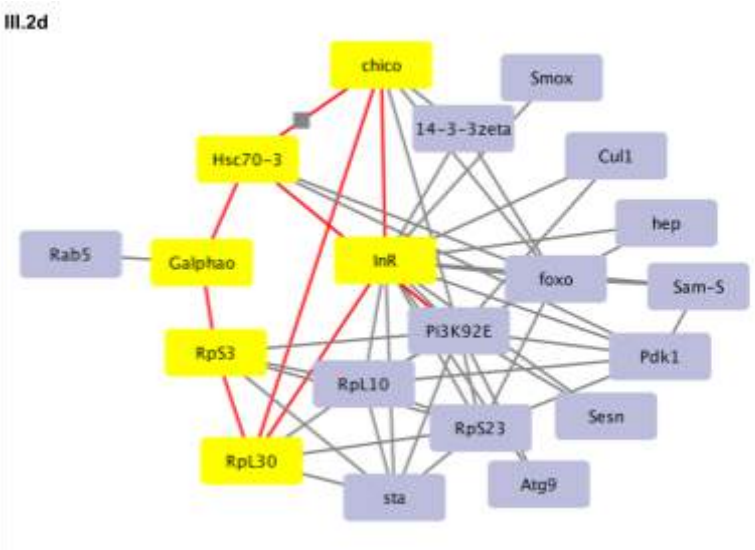


Figure III.2. Overview of Longevity-Associated Gene (LAG) Networks in *Drosophila melanogaster* (a) the full *Drosophila* interactome, (b) *C. elegans* ortholog LAGs, (c) *Drosophila*-specific LAG combinations from SynergyAge, and (d) first-degree interactions of *GαO* and *InR*.

Next, I examined the lifespan of *Drosophila melanogaster* mutants *GαO*, *InR*, and their double mutant *GαO; InR* in comparison to the Canton-S wild-type control (Figure III.3). The lifespan of the Canton-S control reached a maximum of 42 days. In contrast, the *InR* mutant demonstrated a moderate increase to 62 days (Figure III.3a; $p < 0.0001$), while the *GαO* mutant showed a more substantial increase, with a maximum lifespan of 72 days (Figure III.3b; $p < 0.0001$). Notably, the *GαO; InR* double mutant had a lifespan of 65 days, intermediate between the single mutants but shorter than that of *GαO* alone (Figure III.3c; $p < 0.0001$).

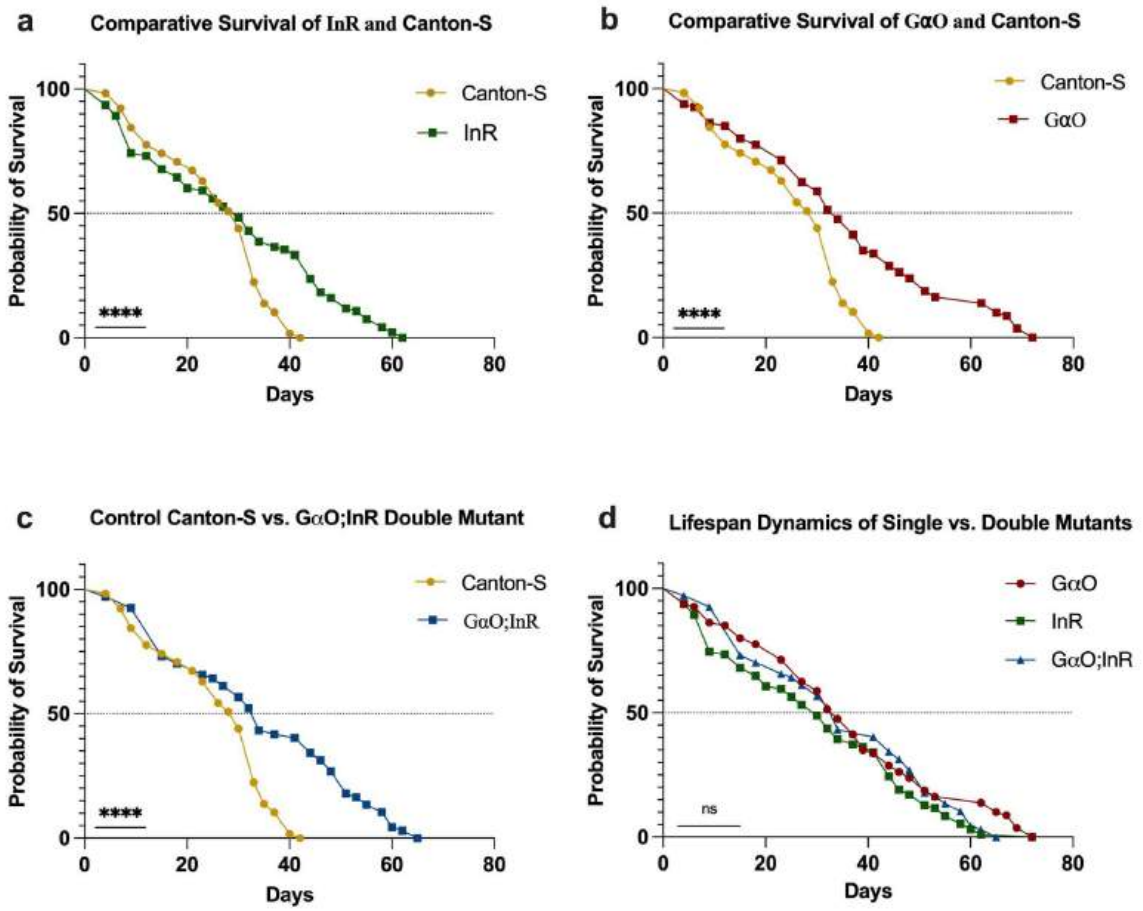


Figure III.3. Survival Curves and Comparative Lifespan Analysis

(a) Lifespan of Canton-S and the *InR* mutant; (b) Comparative lifespan of Canton-S and the *GαO* mutant; (c) Lifespan comparison between Canton-S and the *GαO; InR* double mutant; (d) Survival comparison among *InR*, *GαO* single mutants, and the *GαO; InR* double mutant. This figure was previously published in Romanian Biotechnological Letters, currently in press.

Statistical analyses revealed significant lifespan differences between both InR and GαO single mutants and the Canton-S control ($p < 0.0001$), as well as between the GαO; InR double mutant and control. While both single mutants exhibited lifespan extension, the GαO mutant lived longer than the InR mutant ($p = 0.0145$). However, there was no significant difference between the GαO single mutant and the GαO; InR double mutant ($p > 0.05$), indicating that combining these mutations does not enhance lifespan further, suggesting a dependent rather than synergistic interaction.

The GαO mutation extended lifespan to 72 days, pointing to the role of G-protein-coupled receptor (GPCR) signaling in longevity, a pathway known to regulate neuronal activity and metabolism (*Krishnan et al., 2024; Devambez et al., 2013*). The InR mutant, with a lifespan of 62 days, aligns with the insulin/IGF-1 signaling (IIS) pathway, a well-known regulator of aging (*Bai et al., 2013; Kaletsky and Murphy, 2010*). The GαO; InR double mutant (65 days) did not show additional lifespan extension over GαO alone, suggesting overlapping or dependent mechanisms rather than independent effects.

The lack of a synergistic effect is consistent with patterns in the SynergyAge database, which suggests that non-additive interactions are common in aging-related pathways. This finding indicates that IIS and GPCR signaling may converge on shared downstream targets, explaining the absence of an additive lifespan effect. Given that both pathways influence metabolism, stress response, and neuronal signaling, their interaction might involve shared regulatory mechanisms.

These results contribute to a broader understanding of genetic interactions in aging, highlighting the need to study overlapping pathways. Future research should explore the molecular interplay between GαO and InR and investigate how environmental factors, such as the gut microbiome, might modulate these genetic effects on lifespan.

IV.4. Conclusions

This study highlights how specific genetic pathways interact to influence aging in *Drosophila melanogaster*. By examining single mutants of *GαO* and *InR* alongside their double mutant, we observed that while each gene mutation individually extends lifespan, combining them in the double mutant does not provide any extra longevity benefit beyond what *GαO* alone achieves. This suggests that these genes may act on overlapping pathways with a shared limit on lifespan extension, particularly involving the IIS and GPCR signaling pathways.

The network analyses, supported by SynergyAge data, reveal that such non-additive effects are a common feature in aging pathways, suggesting that genetic interactions in lifespan regulation are more complex and interconnected than a simple additive model. These findings deepen our understanding of how *Drosophila* genes contribute to aging and underscore the importance of looking further into the molecular connections between *GαO* and *InR*. Future research should also explore how external factors, like the gut microbiome, might interact with these genetic pathways to influence lifespan, potentially opening new avenues for aging research.

IV. GENETIC INTERVENTIONS IN DROSOPHILA: ROLE OF PARKIN GENE IN MICROBIOME AND AGING

Acknowledgement: These findings are part of a manuscript ready for submission, based on research conducted at the University of California, Los Angeles (UCLA) within Dr. David Walker's lab in the Department of Integrative Biology and Physiology. This research was supported by a Fulbright Scholarship awarded by the Romanian government.

IV.1. Introduction

The Parkin gene (PARK2), a key regulator of mitophagy and protein degradation, protects against oxidative stress and neurodegeneration (*Kitada et al., 1998; Narendra et al., 2008*). Mutations in PARK2 are linked to early-onset Parkinson's disease (PD) and impair mitochondrial function, accelerating aging and disease progression (*Clark et al., 2006*). While PD has been associated with gut microbiome alterations (*Khedr et al., 2021*), the impact of Parkin overexpression on microbiome composition remains unexplored.

This study examined Parkin overexpression in *Drosophila melanogaster* using daGS>UAS-Parkin flies across days 10, 30, 45, and 60. 16S rRNA sequencing revealed that Parkin modulates microbiome composition in an age-dependent manner. Older control flies (day 60) had higher microbial loads (Acetobacter, Lactobacillus) compared to Parkin-overexpressing flies, while younger Parkin-overexpressing flies (day 10) showed increased Acetobacter, Enterobacter, and Lactobacillus (Fig. IV.2).

Further analysis showed that Parkin-overexpressing flies had fewer bacterial species by day 45, with Commensalibacter stabilizing gut microbiota (Fig. IV.4). Beta diversity analysis confirmed Parkin's influence on microbiome structure, particularly in fermentation-related genera (Fig. IV.11 & 12).

Microbiome transplants into germ-free Canton-S flies showed that older control microbiomes (days 30, 60) reduced lifespan, while Parkin-overexpressing microbiomes maintained stability (Fig. IV.13). Additionally, AMP analysis revealed that control microbiomes induced inflammatory responses, whereas Parkin-overexpressing microbiomes prevented immune activation (Fig. IV.14).

These findings suggest Parkin overexpression shapes microbiome composition, reducing age-related inflammatory shifts and preserving microbial stability, highlighting a potential role in aging regulation beyond mitochondrial quality control.

IV.2. Methods

This study explored the impact of parkin gene overexpression and aging on the gut microbiome, lifespan, and immune responses in *Drosophila melanogaster*. The experimental workflow is summarized in Figure IV.1., which outlines the two main experimental components: midgut sampling for microbiome analysis, and lifespan and immune response assessment.

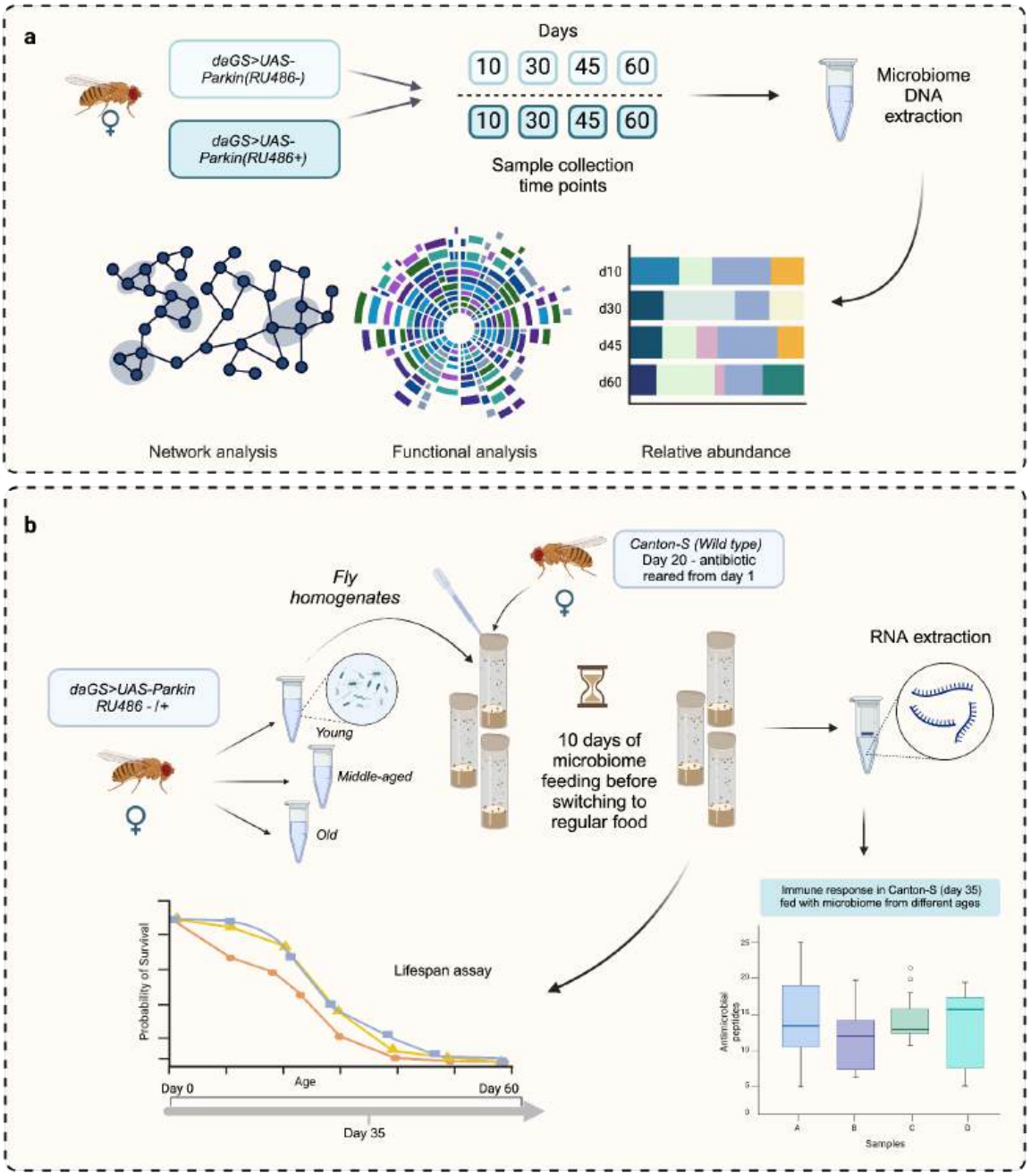


Figure IV.1. Experimental Workflow Diagram. (a) Midgut Dissections and Microbiome Analysis: Illustration of the midgut sampling from Control (Uninduced) and *Parkin* Overexpressed (Induced) *Drosophila melanogaster* at different ages (days 10, 30, 45, and 60), followed by microbial DNA extraction, relative abundance analysis, metagenomic sequencing, and network analysis of microbiome-associated genes. **(b)** Lifespan and Immune Response Assessment: Depiction of the lifespan assay and AMP (antimicrobial peptide) assessment, where germ-free *Canton-S* flies, after microbiome reintroduction from different aged samples, undergo lifespan measurement and AMP level

analysis post-RNA extraction on day 35. Created in BioRender. Sarghie, L. (2024) https://BioRender.com/q02f682

This study used Canton-S wild-type and *daGS>UAS-Parkin* flies, with Parkin overexpression induced via the RU486 GeneSwitch system. Flies were reared on cornmeal-based media, and three-day-old females were synchronized for experiments. Lifespan was assessed by administering RU486 (5 µg/ml), with ethanol-treated controls. Flies were kept in groups of 27–32 per vial, transferred every 2–3 days, and monitored until death.

For microbiome reassociation, axenic Canton-S flies were generated via a 20-day antibiotic treatment, confirmed by culture tests. Microbiomes from *daGS>UAS-Parkin* flies (young, middle-aged, old; RU486+/-) were reintroduced, and flies were maintained for 10 days before returning to standard food.

Microbiome composition was analyzed via 16S rRNA sequencing. DNA was extracted from dissected midguts, and the V4 region was sequenced on Illumina MiSeq. Bioinformatic analysis (DADA2, Picrust2) provided taxonomic and functional insights. qPCR quantified microbial abundance (total 16S, *Lactobacillus, Acetobacter, Enterobacter*), and immune response was assessed via AMP gene expression (Drosomycin, Diptericin), normalized to *Actin 5C*.

Statistical analyses included alpha diversity (phyloseq), beta diversity (Bray-Curtis, PCoA), and differential abundance (DESeq2). PERMANOVA and ANOVA tested diversity changes. Metagenomic predictions used DeepNOG and COGdb on metagenome-assembled genomes (MAGs) to analyze functional shifts under Parkin overexpression.

IV.3. Results

Parkin overexpression modulates gut microbiome composition across different life stages in *Drosophila*, influencing the abundance of key bacterial genera over time. qPCR analysis of total 16S rRNA, Lactobacillus, Acetobacter, and Enterobacter at days 10, 30, 45, and 60 compared microbiome dynamics between control (RU486-) and Parkin-overexpressing (RU486+) flies (Fig. IV.2a-d).

Results showed an age-dependent effect of Parkin overexpression. In young flies (day 10, Fig. IV.2c, d), microbial abundance was higher in the Parkin-overexpressing group, suggesting an early-life role in supporting microbial diversity and gut homeostasis. By middle age (day 30), control flies exhibited increased levels of Enterobacter and

Lactobacillus, indicating a transitional phase in microbiome regulation. In older flies (day 60, Fig. IV.2a, b, d), control flies retained a higher abundance of Acetobacter and Lactobacillus, while Parkin-overexpressing flies showed a reduction, suggesting that Parkin overexpression alters the gut environment, limiting bacterial persistence in aging hosts.

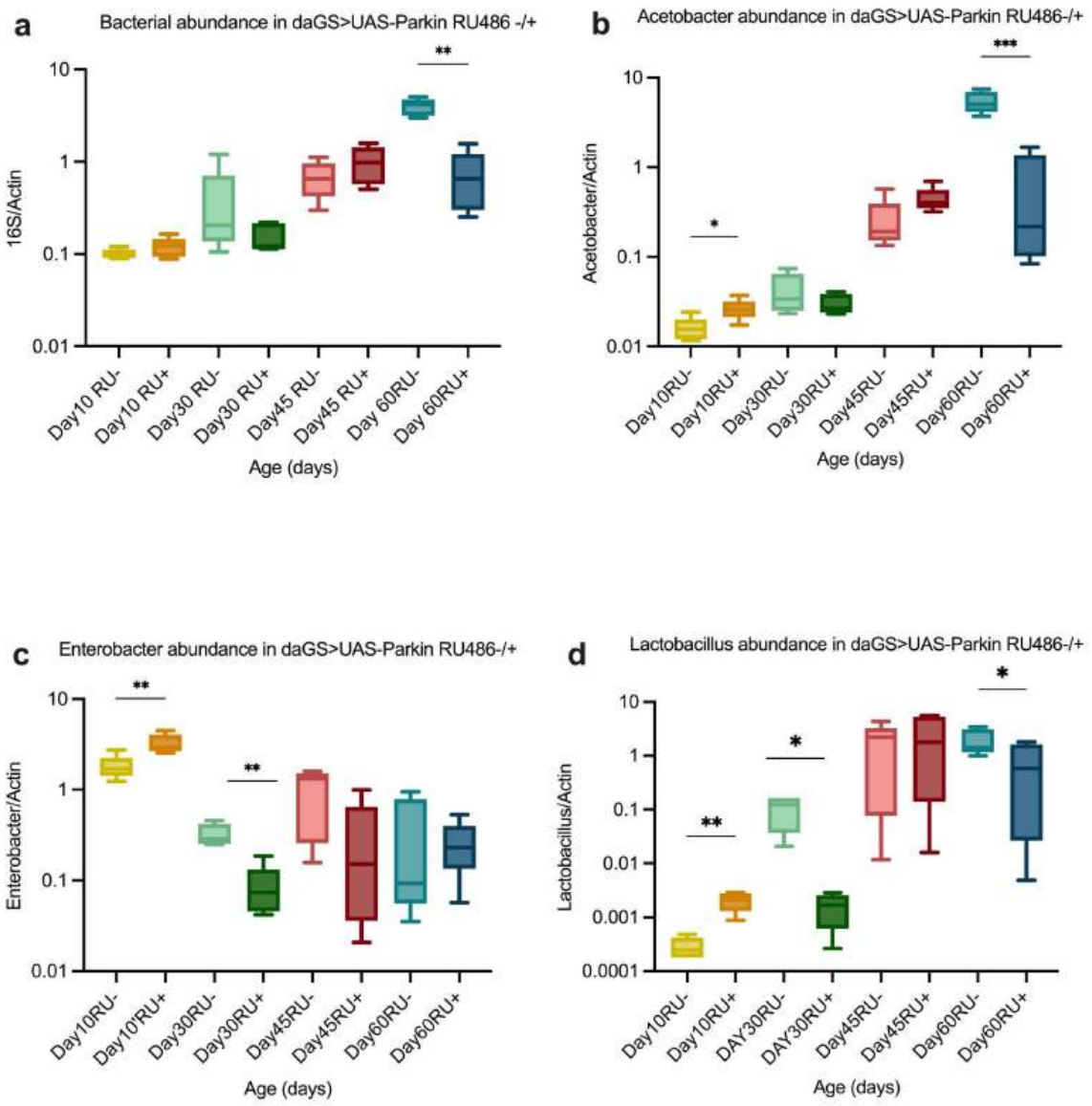


Figure IV.2. Quantification of Bacterial Abundance in *Parkin* Overexpressing and Control Flies Across Ages. Bacterial abundance was measured using qPCR for (a) total *16S gene, (b) Acetobacter, (c) Enterobacter, and (d) Lactobacillus*. Samples were collected at four time points (days 10, 30, 45, and 60) from the *daGS>UAS-Parkin* strain, under two conditions: *parkin* overexpression (induced) and control (uninduced). *Parkin* overexpression was activated from day 1 of life.

To confirm that the microbiome changes observed were a direct result of *parkin* overexpression and not an artifact of RU486 treatment, a control strain was generated by crossing the W1118 line with the Ligand-Bound GeneSwitch driver (daGS). This W1118/daGS control strain was subjected to the same RU486 treatment protocol used to induce *parkin* expression in the experimental groups. Samples from the W1118/daGS control flies were collected at matched time points (days 10, 30, 45, and 60) to provide a reliable basis for comparison with the *parkin*-induced samples (Figure IV.3.).

The results from this control indicated no significant difference in microbiome composition between untreated (uninduced) and RU486-treated (induced) flies, confirming that RU486 treatment alone did not alter microbiome structure. However, a subtle, age-related trend in microbiome composition was observed in the W1118/daGS control group, reflecting a natural shift in microbial populations as the flies aged. These findings suggest that, while aging impacts the gut microbiota composition, the specific microbiome shifts observed in the experimental group can be attributed to *parkin* overexpression rather than any unintended effects of RU486. This control approach strengthens the conclusion that *parkin* expression uniquely influences gut microbial dynamics across different ages.

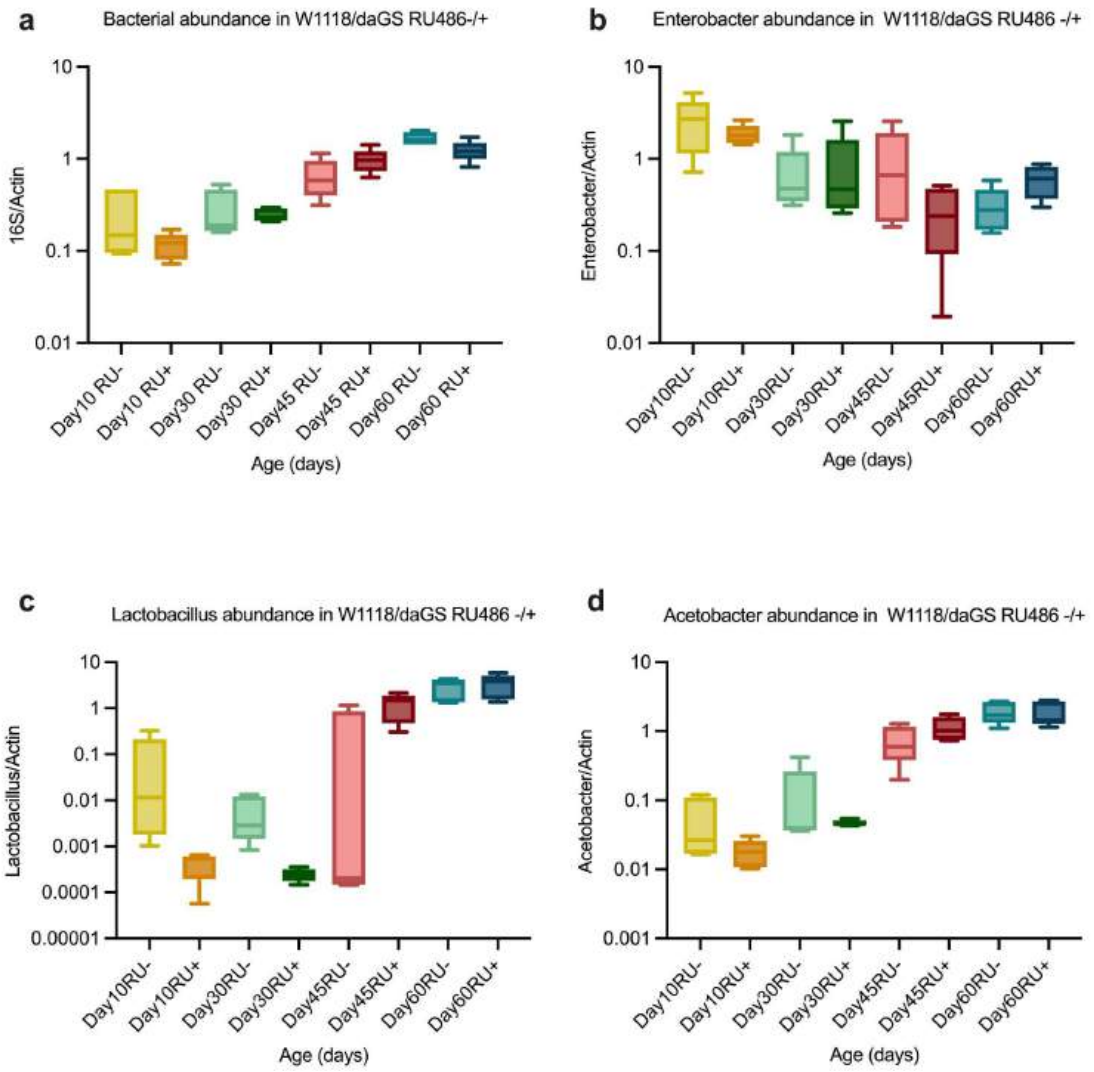


Figure IV.3. Microbiome Composition in W1118 control and treated (RU486). Bacterial abundance was measured via qPCR for (a) total 16S gene, (b) *Acetobacter*, (c) *Enterobacter*, and (d) *Lactobacillus*. Samples were collected from the W1118/daGS control strain at four time points (days 10, 30, 45, and 60) under both control (untreated) and RU486-treated conditions.

Alpha diversity was assessed using Observed Richness, Shannon Diversity, and Inverse Simpson Diversity to compare Whole Genome Sequencing (WGS) and 16S rRNA sequencing.

16S rRNA sequencing detected more unique taxa, particularly in low-abundance samples (e.g., S01: 94 vs. 76 taxa, S08: 71 vs. 7 taxa), highlighting its greater sensitivity to rare taxa. Shannon and Inverse Simpson Diversity indices showed that WGS identified more evenly distributed microbial communities in early samples (S01, S02), while 16S

revealed greater diversity in later samples (S07, S08), suggesting WGS captured dominant taxa, whereas 16S provided a broader taxonomic profile.

These results emphasize the complementary nature of both methods: 16S excels at detecting rare taxa, while WGS offers a more balanced view of community structure, making the choice dependent on specific research objectives.

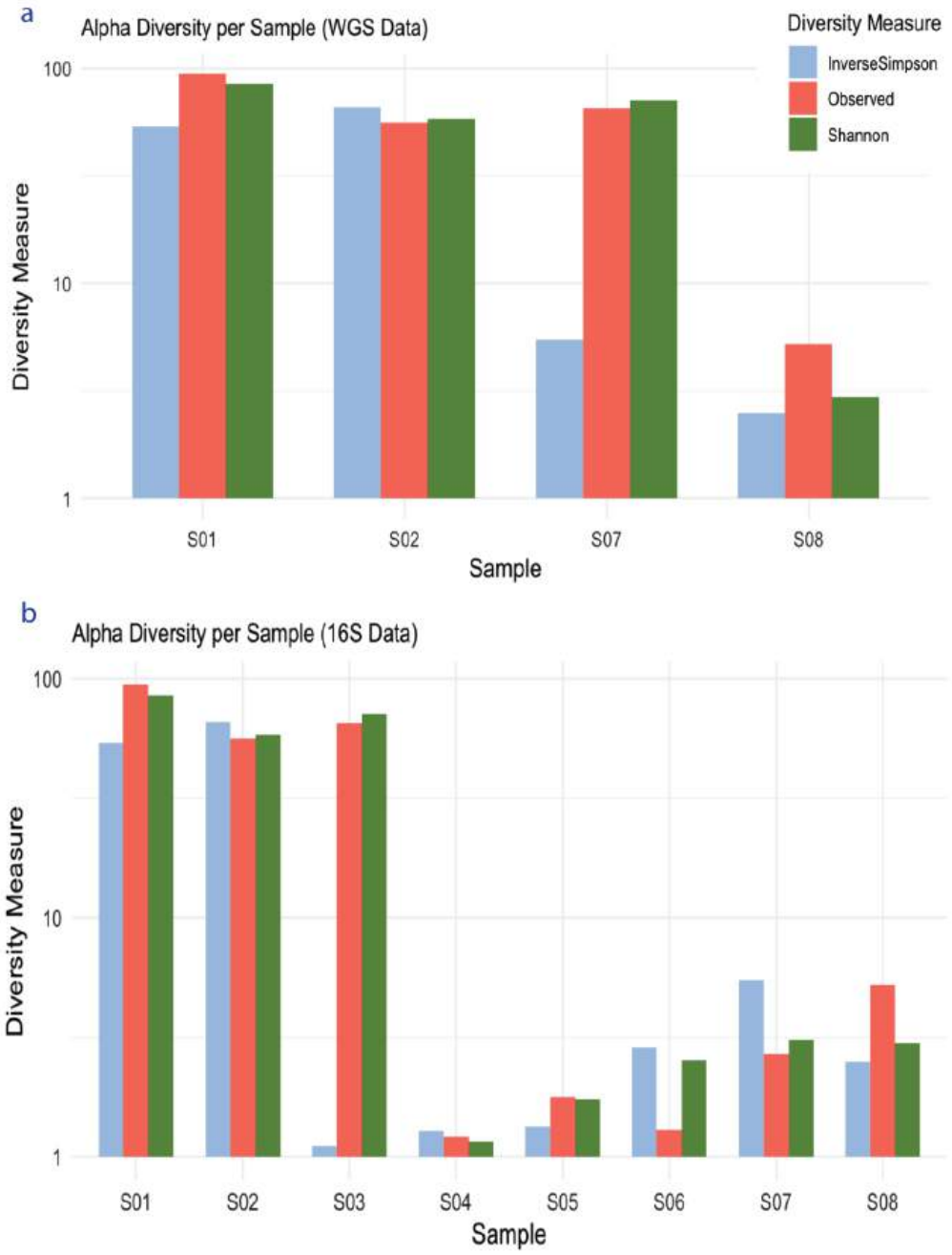


Figure IV.5. Alpha diversity metrics derived from Whole Genome Sequencing (WGS) and 16S rRNA gene sequencing across samples. Panels (a) and (b) showcase Observed richness, Shannon, and Inverse Simpson indices for WGS and 16S data, respectively, providing a comparative analysis of microbial alpha diversity.

To better understand the differences in microbial communities, we analyzed the relative abundance of the 25 most prevalent bacterial taxa using both Whole Genome Sequencing (WGS) and 16S rRNA sequencing (Fig. IV.6.(a) and (b), respectively). These analyses highlight distinct microbial profiles across samples and demonstrate how each sequencing approach captures community composition differently.

In the WGS dataset (Fig. IV.6.a), *Acetobacter pasteurianus* was the dominant species in samples S01 and S02, suggesting its strong presence in these microbial environments. On the other hand, *Lactobacillus brevis* was the most abundant taxon in sample S08, distinguishing it from the other samples. Interestingly, the 16S rRNA sequencing results (Fig. IV.6.b) also identified *Lactobacillus brevis* as a major component in S08, indicating a consistent pattern across both sequencing methods. This suggests that sample S08 has a distinct microbial signature, where *Lactobacillus brevis* plays a significant ecological role.

The dominance of *Lactobacillus brevis* in S08 could be influenced by environmental factors, host interactions, or other selective pressures that support its growth in this sample. The agreement between WGS and 16S data for this taxon strengthens confidence in its biological relevance.

Beyond individual species, these findings reveal clear differences in microbial composition across samples, reflecting how WGS and 16S sequencing provide complementary perspectives on community diversity. WGS captures a broader functional profile by sequencing entire genomes, while 16S remains effective for taxonomic classification, particularly for dominant bacterial groups. These results highlight the impact of sequencing methodology on microbiome studies and emphasize the importance of interpreting findings within the strengths and limitations of each approach.

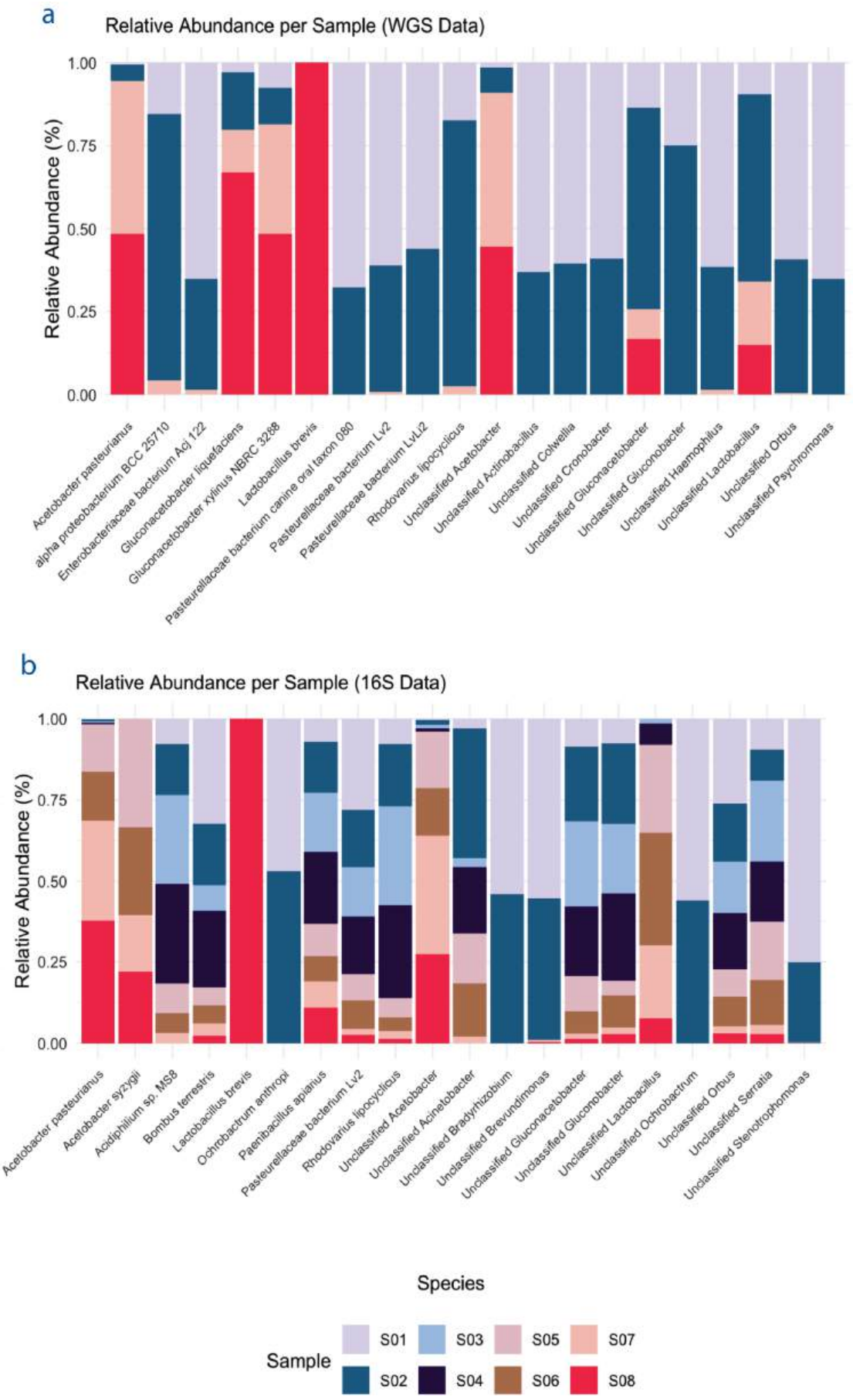
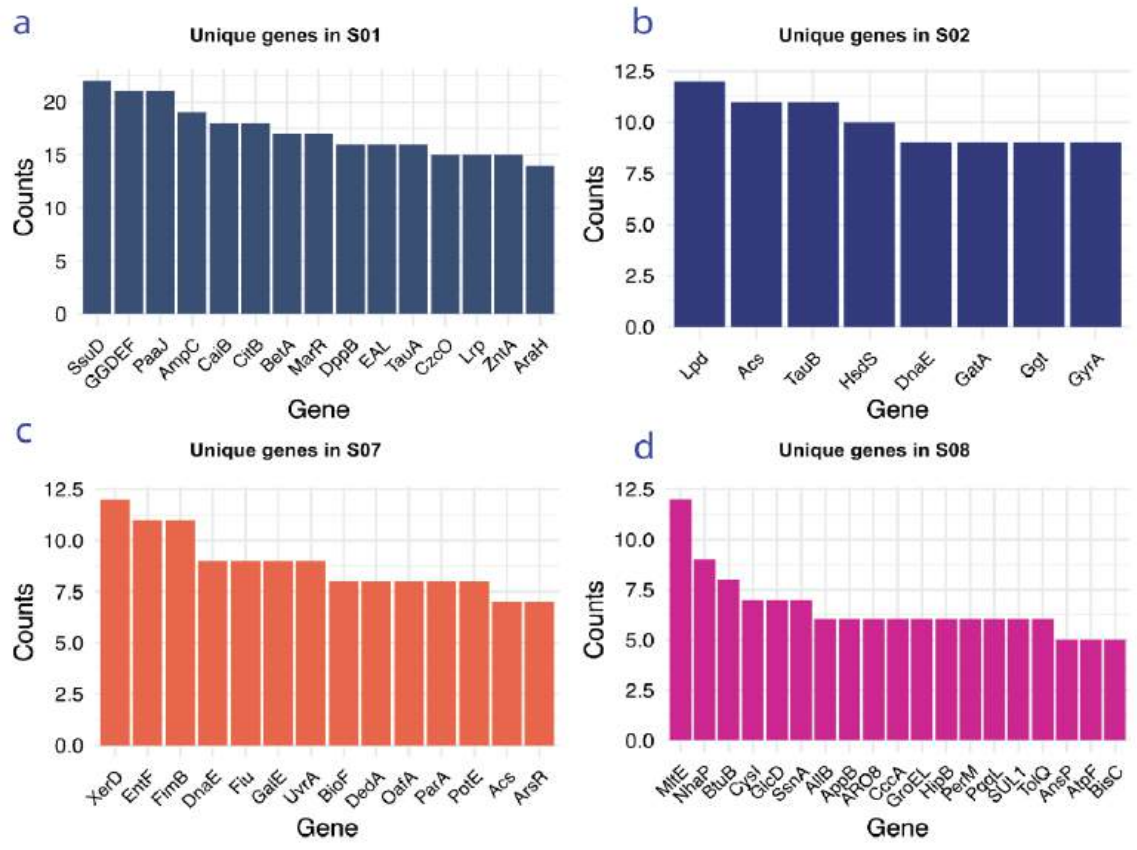

a
Relative Abundance per Sample (WGS Data)
Relative Abundance (%)
1.00
0.75
0.50
0.25
0.00
Acetobacter pasteurianus
alpha proteobacterium BCC 25710
Enterobacteriaceae bacterium Acj 122
Gluconacetobacter liquefaciens
Gluconacetobacter xylinus NBRC 3288
Lactobacillus brevis
Pasteurellaceae bacterium canine oral taxon 080
Pasteurellaceae bacterium Lv2
Pasteurellaceae bacterium LvL2
Rhodovarius lipocyclicus
Unclassified Acetobacter
Unclassified Actinobacillus
Unclassified Colwellia
Unclassified Cronobacter
Unclassified Gluconacetobacter
Unclassified Gluconobacter
Unclassified Haemophilus
Unclassified Lactobacillus
Unclassified Orbus
Unclassified Psychromonas
b
Relative Abundance per Sample (16S Data)
Relative Abundance (%)
1.00
0.75
0.50
0.25
0.00
Acetobacter pasteurianus
Acetobacter syzygii
Acidiphilium sp. MS8
Bombus terrestris
Lactobacillus brevis
Ochrobactrum anthropi
Paenibacillus apiarius
Pasteurellaceae bacterium Lv2
Rhodovarius lipocyclicus
Unclassified Acetobacter
Unclassified Acinetobacter
Unclassified Bradyrhizobium
Unclassified Brevundimonas
Unclassified Gluconacetobacter
Unclassified Gluconobacter
Unclassified Lactobacillus
Unclassified Ochrobactrum
Unclassified Orbus
Unclassified Serratia
Unclassified Stenotrophomonas
Species
Sample
S01
S02
S03
S04
S05
S06
S07
S08

Figure IV.6. Relative abundance of the 25 most prevalent bacterial taxa identified through Whole Genome Sequencing (WGS) and 16S rRNA gene sequencing. Panels (a) and (a) display the relative abundance (%) of the 25 most prevalent bacterial taxa, identified through Whole Genome Sequencing (WGS) and 16S rRNA gene sequencing, respectively. Taxa are color-coded according to the legend for clear visualization.

WGS data identified 50 unique age-related genes, with distinct metabolic roles at each stage. At Day 10, genes linked to sulfur metabolism (SsuD), signaling (GGDEF), and ion transport (ZntA) were predominant. By Day 30, metabolic functions shifted toward oxidative metabolism (LpdA, Acs) and DNA replication (DnaE, GyrA). At Day 45, genes related to DNA repair (XerD, UvrA) and heavy metal resistance (ArsR) indicated adaptation to environmental stress. By Day 60, the microbiome prioritized cell wall maintenance (MltE), ion homeostasis (NhaP), protein folding (GroEL), and ATP synthesis (AtpF), suggesting a focus on cellular stability and energy production in late adulthood.

These findings illustrate how microbial metabolism dynamically adapts to aging, with early-stage communities emphasizing growth and signaling, while older microbiomes shift toward stress resilience and maintenance.



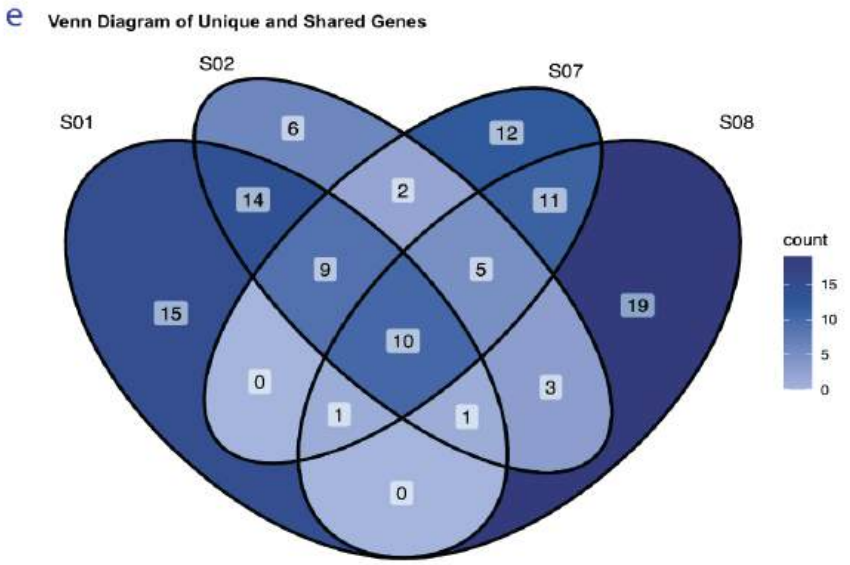


Figure IV.10. Metabolic Function Prediction Across Samples. Panels (a)–(d) highlight the top 10 unique genes identified in each WGS sample, revealing key metabolic functions such as sulfur metabolism, DNA repair, energy production, and stress response. These findings suggest that microbial communities undergo functional adaptations at different ages. Panel (e) presents a Venn diagram illustrating the distribution of shared and unique genes across samples. This visualization highlights core genes that are common to multiple samples, as well as unique gene sets that reflect distinct metabolic requirements in each microbial community.

While Parkin overexpression extends lifespan and supports mitochondrial function in *Drosophila*, its effects on the gut microbiome and host longevity remain unclear. This study examined how microbiomes from Parkin-overexpressing (OE) and control flies influence germ-free hosts.

Canton-S flies were made germ-free via a 20-day antibiotic treatment, which had no impact on lifespan. After microbiome depletion, flies were exposed to microbiomes from control or Parkin-OE flies at days 10, 30, and 60. Lifespan analysis showed no difference for young (day 10) microbiomes, but middle-aged (day 30) and old (day 60) control microbiomes significantly shortened lifespan. In contrast, flies receiving microbiomes from Parkin-OE donors maintained longevity across all ages.

These findings suggest Parkin overexpression preserves microbiome stability, preventing aging-associated microbial shifts that negatively impact lifespan.

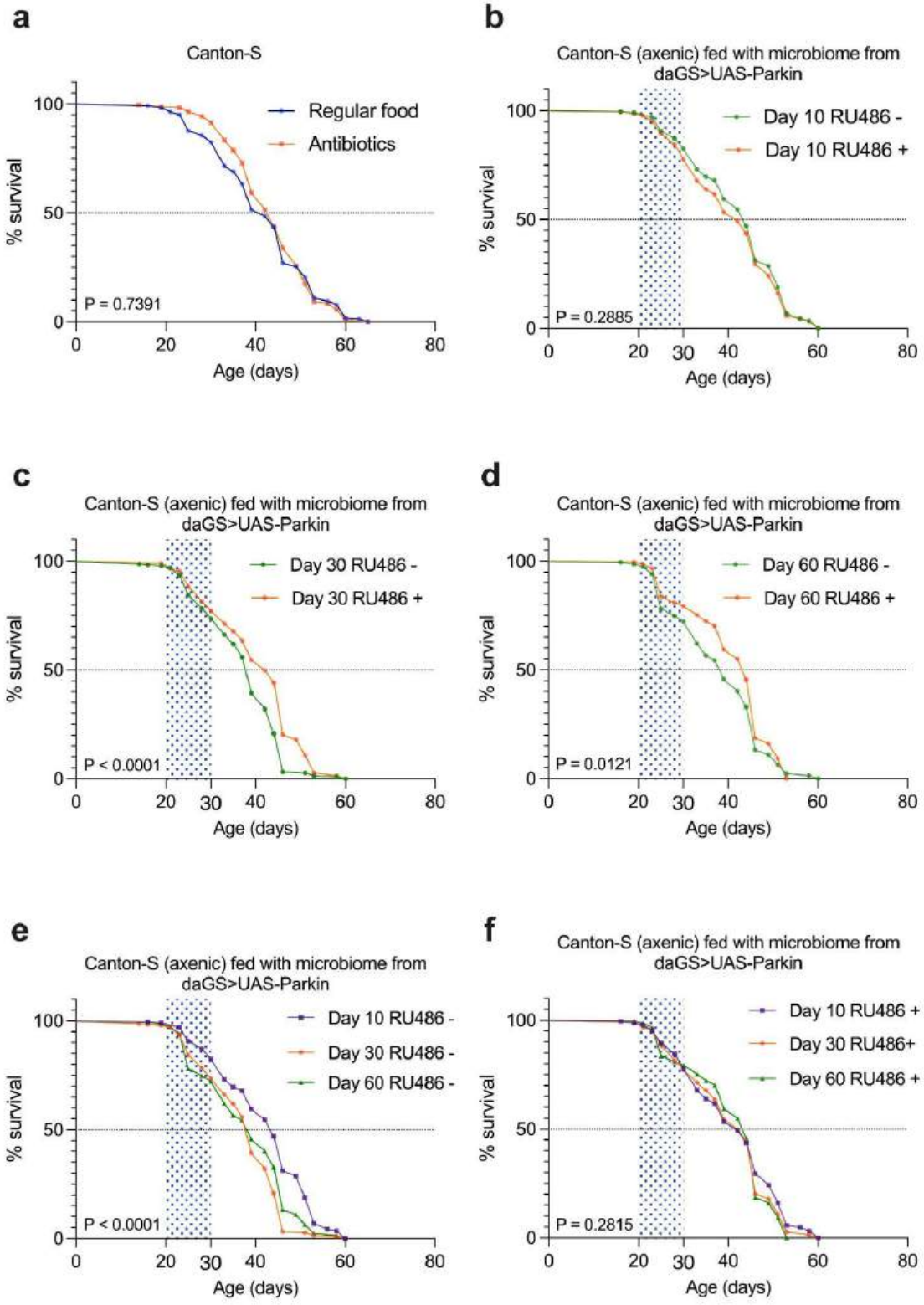


Figure IV.13. Lifespan assay of *Canton-S* flies following microbiome reintroduction from control and induced *parkin* flies at different ages. This figure shows the lifespan assay of axenic Canton-S flies, reared on antibiotics for 20 days before

being reintroduced to microbiomes from control (uninduced) and Parkin-induced flies at days 10, 30, and 60, then maintained on standard food. (a) Verifies that antibiotic treatment alone does not affect lifespan. (b-d) Survival curves comparing flies reintroduced to control vs. induced microbiomes at young (10d), middle-aged (30d), and old (60d) stages. (e-f) Lifespan comparisons of flies receiving control vs. induced microbiomes across all age groups.

We examined how microbiome transfer influenced immune response in *Canton-S* flies. After 10 days of microbiome feeding (days 20–30), flies were switched to a regular diet for five days before RNA extraction on day 35. Expression of antimicrobial peptides *Drosomycin* and *Diptericin* was measured to assess immune activation.

Flies fed microbiomes from young (day 10) and middle-aged (day 20) *daGS>UAS-Parkin* flies showed similar AMP expression under both control and induced conditions. However, those receiving microbiomes from old (day 60) control flies had significantly higher *Drosomycin* and *Diptericin* levels than those given microbiomes from old *Parkin*-induced flies, suggesting pro-inflammatory changes in aging microbiomes.

A similar pattern was observed in *Canton-S* flies fed their own age-matched microbiomes. Those fed middle-aged (day 20) microbiomes showed higher AMP expression than those fed young (day 10) microbiomes, indicating that microbiome aging may drive immune activation. These findings suggest that age-associated microbiome shifts can induce inflammation, with control flies' microbiomes more likely to trigger immune activation than those from *Parkin*-induced flies.

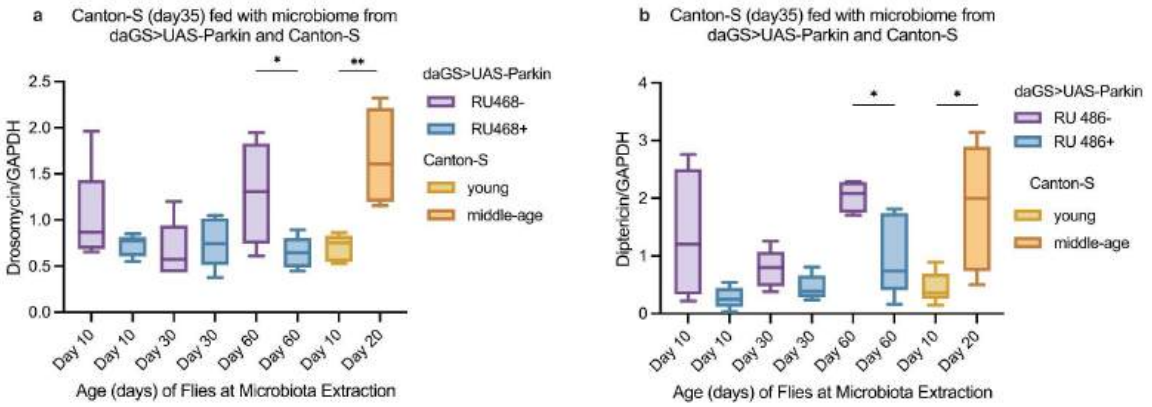


Fig. IV.14. Antimicrobial Peptide Assay. Assessment of Drosomycin (a) and Diptericin (b) levels in Canton-S flies following microbiome manipulation.

IV.5. Conclusions

In this study, I investigated the impact of *parkin* overexpression on the gut microbiome composition in *Drosophila melanogaster* and its implications for host health across different life stages. By conducting a comparative analysis between *parkin*-induced and control flies over a 60-day period, I was able to observe how *parkin* expression modulates specific bacterial genera as the host ages. My approach included quantifying microbial abundance through qPCR for total 16S rRNA as well as specific bacterial taxa, such as *Lactobacillus*, *Acetobacter*, and *Enterobacter*, across multiple age points. The findings reveal that *parkin* overexpression dynamically influences the gut microbiome, enhancing microbial diversity in younger flies while reducing the abundance of certain bacteria in older flies. This age-dependent microbial shift suggests that *parkin* may play a regulatory role in maintaining a balanced gut environment, fostering beneficial microbial interactions during early life stages and selectively reducing specific bacterial populations with age.

Furthermore, through microbiome transplant experiments, I assessed how these shifts in microbial composition affect lifespan and immune response in germ-free Canton-S flies. By reintroducing microbiomes from both *parkin*-induced and control flies into antibiotic-treated, axenic Canton-S flies, I demonstrated that microbiomes from aged control flies had a marked pro-inflammatory effect and reduced host lifespan. In contrast, the microbiome from *parkin*-induced flies did not trigger similar declines, underscoring the potential protective effect of *parkin* on gut microbial composition and host longevity.

The study also highlights the diversity of microbiome compositions across samples, with Lactobacillus brevis emerging as a key taxon in S08. The consistency of WGS and 16S results for L. brevis supports its ecological relevance in this sample, reinforcing the robustness of our sequencing approach. Interestingly, the presence of L. brevis in S08 aligns with previous findings in C. elegans models, where this bacterium has been associated with enhanced stress resistance and lifespan extension (Thiruppathi et al. 2023; Kumar et al., 2022). The mechanisms underlying these effects, including modulation of oxidative stress and longevity-related signaling pathways, suggest that microbial composition may influence aging-related processes, potentially through interactions with the host's metabolic and immune systems.

Additionally, I observed changes in microbial alpha and beta diversity that corresponded with *parkin* overexpression. These shifts, particularly the role of *Commensalibacter*, which was prevalent in the gut microbiome of younger flies, suggest

that *parkin* may influence not only microbial composition but also the functional capabilities of the gut microbiome over time. The distinct metabolic pathways associated with the *parkin*-induced microbiome, such as *menaquinol-7* biosynthesis and nicotinate degradation, highlight the potential impact of *parkin* on host metabolic processes, likely contributing to enhanced energy homeostasis and cellular resilience.

The integration of findings on *L. brevis* further supports the hypothesis that microbial composition plays a crucial role in aging-related processes. While direct extrapolation to *Drosophila melanogaster* requires further validation, the geroprotective properties of *L. brevis* observed in *C. elegans* provide compelling evidence that specific microbial taxa may influence host longevity through conserved metabolic and stress-response pathways.

Moreover, this study is the first to explore how *parkin* overexpression influences gut microbial dynamics and its broader implications for host aging and longevity. The results provide valuable insights into the complex interplay between genetic factors and the microbiome, suggesting that *parkin* may regulate gut health and potentially mitigate age-related microbiome changes. Further research with larger sample sizes will deepen our understanding of these interactions, offering a clearer picture of how *parkin*-mediated gut microbiome modulation could be harnessed to support healthy aging.

LIST OF PUBLICATIONS AND PRESENTATIONS

Publications:

1. Bunu G*, Toren D*, Ion CF, Barardo D, **Sârghie L**, Grigore LG, de Magalhães JP, Fraifeld V, Tacutu R. "SynergyAge, a curated database for synergistic and antagonistic interactions of longevity-associated genes", Scientific Data, 7:366 (2020), https://doi.org/10.1038/s41597-020-00710-z, **IF = 5.8 , Q1, AIS = 1.937**

2. **Larisa Sârghie1,** Carmen Tanasa , Ioana Popa , Robi Tacutu. "Functional Analysis of GαO and InR in Regulating Longevity in Drosophila melanogaster", was accepted for publication in Vol. 28, No. 5/2024 of Romanian Biotechnological Letters Journal, **Web of Science BDI**

Poster presentations

1. Sârghie, L. Aparicio, R., Walker, D.W. "Exploring the interplay between mitochondrial homeostasis, microbiota dynamics and aging", **Aging Research and Drug Discovery**, Denmark, August 2023 - poster

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