Synergistic Glucose Depletion in *Tenebrio Molitor* Gut by a Multi-strain Bacterial Consortium

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Abstract

The gut microbiome is a critical regulator of host metabolism, and disruptions to microbial composition are increasingly linked to metabolic disorders. Marine sponges harbor metabolically versatile symbiotic bacteria, yet their potential to modulate host glucose regulation remains unexplored. Here, we investigated whether sponge-analog bacterial strains influence glucose metabolism in *Tenebrio molitor*. Adult worms were colonized for 72 hours with *Vibrio natriegens*, *Streptomyces griseus*, *Micrococcus luteus*, *Bacillus megaterium*, or a five-strain consortium, each co-introduced with *Escherichia coli*. Gut glucose levels were quantified using the Glucose Oxidase–Peroxidase (GOD–POD) assay. Bacterial treatment had a significant effect on glucose concentration (ANOVA, $F_{7,40} = 187.74$, $p < 0.0001$). Single-strain treatments reduced glucose by 25–32% relative to uncolonized controls, whereas the five-strain consortium produced a markedly greater at 53% reduction. Heat-killed controls showed minimal change, confirming dependence on live microbial metabolism. These findings demonstrate that defined microbial communities inspired by sponge-associated taxa can substantially modulate host glucose availability, with multi-strain synergy producing the strongest metabolic effects. This work highlights *T. molitor* as a tractable model for host–microbiome metabolic interactions and supports further exploration of engineered bacterial consortia for probiotic metabolic modulation.

Keywords: *Gut microbiome, Glucose metabolism, Tenebrio molitor, Bacterial consortium, Host-microbe interaction, Metabolic modulation, Invertebrate model*

Introduction

The gut microbiome, a complex and dynamic community of microorganisms inhabiting the gastrointestinal tract, plays a central role in regulating digestion, immune defense, and host metabolism. Through the production of metabolites such as short-chain fatty acids (SCFAs) and bile acids, gut bacteria influence host glucose homeostasis, lipid metabolism, and inflammation [1]. When the microbial balance is disturbed—a condition known as dysbiosis—these host–microbe interactions are disrupted, contributing to a variety of chronic disorders including obesity, insulin resistance, and type 2 diabetes mellitus (T2D) [2,3].

T2D, characterized by chronic hyperglycemia and reduced insulin sensitivity, is one of the most prevalent metabolic diseases worldwide. While genetic and lifestyle factors are well-established determinants, mounting evidence indicates that the gut microbiota acts as an independent modulator of host metabolism. Certain bacterial taxa have been linked to improved glycemic control through enhanced glucose uptake and SCFA production, whereas others promote metabolic inflammation and insulin resistance by impairing intestinal barrier integrity [2]. These findings highlight the need for tractable model systems that can isolate and experimentally test defined microbial influences on host physiology.

Invertebrate systems have emerged as valuable models for microbiome research due to their simplicity, short life cycles, and well-characterized gut environments. *Tenebrio molitor* (mealworm beetle) is particularly suited for metabolic microbiology because of its large body size, low-complexity gut microbiota, and compartmentalized digestive tract that mirrors mammalian intestinal organization. Its dominant bacterial phyla—Firmicutes and Proteobacteria—parallel those of the human gut, and germ-free or gnotobiotic individuals can be established to evaluate direct causal effects of specific microbes [4]. Together, these features make *T. molitor* a practical model for elucidating host–microbe metabolic interactions.

Marine sponges provide another compelling example of complex symbiotic microbiomes. Accounting for up to 40 % of sponge biomass, sponge-associated bacterial communities are highly diverse and metabolically active, producing a wide spectrum of secondary metabolites involved in nutrient cycling, chemical defense, and oxidative balance [5]. Genera such as *Vibrio*, *Bacillus*, *Streptomyces*, and *Micrococcus* are abundant within these systems and are known to synthesize glycosidase inhibitors, peptides, and antioxidants—molecules with potential to influence host glucose metabolism and insulin sensitivity [6,7]. Despite extensive biochemical characterization of these symbionts, their capacity to modulate host metabolic processes remains poorly understood.

To explore this potential, we modeled sponge-derived microbial consortia using four representative bacterial strains: *Vibrio natriegens*, *Streptomyces griseus*, *Micrococcus luteus*, and *Bacillus megaterium*. These species were selected for their phylogenetic diversity and metabolic versatility—traits that mirror functional roles within marine sponge microbiota. *V. natriegens* is a fast-growing marine bacterium with broad metabolic adaptability [8], *S. griseus* and *M. luteus* produce bioactive compounds with antidiabetic or antimicrobial properties, and *B. megaterium* exhibits enzymatic diversity and probiotic potential [6,7].

We hypothesized that colonization of *T. molitor* by these marine sponge–analog bacteria would alter host glucose metabolism through both direct microbial activity and host-mediated signaling. Specifically, this study aimed to (1) determine whether defined bacterial colonization modulates glucose concentrations in *T. molitor*, and (2) evaluate whether multi-strain consortia exert synergistic effects exceeding those of individual strains. By integrating marine microbiology with a tractable insect host model, this research establishes a novel framework for examining microbiome-driven metabolic regulation and identifies marine symbiotic bacteria as promising candidates for metabolic modulation.

Methods

Bacterial Cultivation and Preparation

Four bacterial strains—*Vibrio natriegens*, *Streptomyces griseus*, *Micrococcus luteus*, and *Bacillus megaterium*—were selected as analogs of marine sponge–associated microbiota. All strains were obtained from Carolina Biological Supply and stored at 4 °C until use. Strains were streaked onto Luria–Bertani (LB) agar plates (10 g tryptone, 5 g yeast extract, 10 g NaCl per L; pH 7.0) and incubated aerobically at 25 ± 1 °C for 24 h. *Escherichia coli* ATCC 25922 served as a positive colonization control.

Fresh single colonies were transferred to 5 mL sterile LB broth and grown at 25 °C with shaking (150 rpm) for 16–18 h to reach late-log phase. Bacterial lawns for colonization were prepared by spreading 200 µL of each culture evenly across LB agar plates and allowing them to air-dry for 10 min under a laminar-flow hood. Cell densities were standardized by adjusting each suspension to OD_{600} = 1.0 using sterile LB broth (path length = 1 cm cuvette), corresponding to approximately 10^8 CFU/mL.

For the five-strain consortium, equal volumes (200 µL each) of the five OD-adjusted cultures were combined immediately before plating. Heat-killed *E. coli* controls were prepared by autoclaving standardized suspensions at 121 °C for 15 min and cooling them to room temperature prior to plating.

All media preparation, transfers, and mixing were conducted aseptically inside a Class II biosafety cabinet to prevent contamination.

Worm Rearing and Bacterial Colonization

Adult *Tenebrio molitor* of uniform age (3–4 weeks post-pupation) and size were sourced from a local supplier and maintained on sterilized wheat bran and fresh potato slices at 25 ± 1 °C and 60 ± 5% relative humidity. Worms were acclimated for one week prior to experimentation. To standardize gut contents, individuals were starved for 12 h before colonization.

Worms were randomly assigned to eight treatment conditions (n = 6 worms per group). For colonization, each worm was placed individually onto a sterile 90 mm Petri dish containing a fully confluent bacterial lawn prepared as described above. Plates were sealed with parafilm to

maintain humidity and limit desiccation, then incubated for 72 h at 25 °C in darkness to mimic burrow conditions. For negative controls, worms were placed on sterile LB agar without bacteria; for heat-killed controls, plates contained lawns of autoclaved *E. coli*.

Throughout colonization, no antibiotics were used, and worms were kept in separate plates to prevent cross-contamination. All handling occurred under sterile conditions.

Table 1. Summary of the eight treatment conditions used to assess bacterial effects on glucose metabolism in *Tenebrio molitor*. Each treatment consisted of six biological replicates (n = 6 worms per group), and bacterial compositions were standardized to OD_{600} = 1.0 before colonization.

Treatment Groups	Composition	Purpose
Negative Control	No bacteria	Baseline glucose level
Positive Control	*E. coli* only	Standard colonization control
Negative Control #2	Heat-killed *E. coli*	Non-viable bacteria control
Test #1	*M. luteus* + *E. coli*	Single-strain metabolic effect
Test #2	*B. megaterium* + *E. coli*	Single-strain metabolic effect
Test #3	*V. natriegens* + *E. coli*	Single-strain metabolic effect
Test #4	*S. griseus* + *E. coli*	Single-strain metabolic effect
Test #5	*V. natriegens*, *S. griseus*, *M. luteus*, *B. megaterium*, + *E. coli*	Multi-strain consortium

Dissection and Sample Preparation

After 72 h, worms were cold-anesthetized at 4 °C for 5 min, surface-sterilized by immersion in 70% ethanol for 30 s, rinsed twice in sterile, ice-cold phosphate-buffered saline (PBS; pH 7.4), and transferred to sterile filter paper to dry. All dissections were performed under a stereomicroscope using flame-sterilized fine forceps.

Entire gastrointestinal tracts were removed and placed individually into sterile 1.5 mL microcentrifuge tubes containing 200 µL ice-cold PBS. Samples were homogenized using sterile disposable micropestles for 30 s and vortexed briefly to ensure consistency. Lysates were centrifuged at 1000 × g for 2 min at 4 °C, and supernatants were transferred to fresh tubes for glucose quantification.

Baseline (t = 0) biological replicates (n = 6 worms) were processed identically prior to bacterial exposure. Each biological sample represented one individual worm. Technical replicates (triplicate wells) were performed for each biochemical assay.

Glucose Quantification (GOD-POD Assay)

Glucose concentrations were measured using the Glucose Oxidase–Peroxidase (GOD–POD) Assay Kit (Sigma-Aldrich MAK476; St. Louis, MO, USA), following the manufacturer's protocol (Sigma-Aldrich, 2023). Ten microliters of each gut lysate were added to 100 µL of GOD–POD reagent in a flat-bottom 96-well plate. Plates were incubated for 30 min at 37 °C in the dark, and absorbance was read at 570 nm using a Bio-Rad iMark microplate reader.

A standard curve (0–200 mg/dL D-glucose) was generated for each assay plate. Only standard curves with $R^2 \geq 0.99$ were accepted. Final glucose concentrations (µM) were calculated using linear regression and normalized to the volume of gut lysate extracted.

Statistical Analysis

All statistical analyses were performed using Microsoft Excel and R (version 4.3.0; R Core Team). Data from biological replicates (n = 6 per group) were summarized as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare glucose concentrations across treatment groups. Tukey's Honestly Significant Difference (HSD) post-hoc test assessed pairwise comparisons with $\alpha = 0.001$.

Significance was defined as $p < 0.001$. Figures were generated using the *ggplot2* package (version 3.4.0), and error bars represent standard error of the mean (SE) unless otherwise stated.
Randomization was used to assign worms to treatment groups, and analyses were performed unblinded. All procedures complied with guidelines for invertebrate research, which does not require IACUC approval.

Results

Glucose concentrations and spectrophotometric absorbance values were measured in *Tenebrio molitor* adults following 72 hours of colonization with eight bacterial treatments. Each subsection below presents raw data, descriptive statistics, and inferential statistical findings, organized to reflect the progression from unprocessed measurements to statistical comparisons. A summary of mean absorbance and glucose concentrations, along with standard deviations and standard errors for all treatment groups, is provided in Table 2.

Table 2. Descriptive statistics for absorbance (AU) and glucose concentrations (µM). Means, standard deviations (SD), and standard errors (SE) for the eight treatment groups (n = 6 worms per group). The five-strain consortium showed the lowest mean glucose levels and correspondingly lowest absorbance values.

Treatment Group	AU Mean	AU SD	AU SE	Glucose Mean (µM)	Glucose SD	Glucose SE

Negative Control	1.237	0.051	0.021	274.81	11.30	4.61
Positive Control (*E. coli*)	1.047	0.050	0.020	232.59	11.04	4.51
Test #1 (*M. luteus* + *E. coli*)	0.953	0.038	0.015	211.85	8.39	3.43
Test #2 (*B. megaterium* + *E. coli*)	0.900	0.026	0.011	200.00	5.79	2.37
Test #3 (*V. natriegens* + *E. coli*)	0.867	0.022	0.009	192.59	4.80	1.96
Test #4 (*S. griseus* + *E. coli*)	0.920	0.024	0.010	204.44	5.26	2.15
Test #5 (Five-strain consortium)	0.598	0.023	0.009	132.96	5.15	2.10
Heat-killed *E. coli*	1.135	0.027	0.011	252.22	6.09	2.48

Baseline Absorbance and Glucose Measurements

Baseline measurements were collected from uncolonized worms prior to bacterial exposure in order to establish reference values for both absorbance (AU) and glucose concentration. Absorbance values ranged from 1.277 to 1.379 AU across the six individuals, with a mean of 1.325 AU, reflecting a narrow distribution of initial endogenous glucose levels. Corresponding glucose concentrations, calculated from the MAK476 standard curve, ranged from 283.8 to 305.8 µM. These values indicate that all worms began the experiment with comparable metabolic states.

Standard deviation (SD) and standard error of the mean (SE) were calculated using conventional formulas:

$$SD = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$$

$$SE = \frac{SD}{\sqrt{n}}.$$

As an example, the negative control absorbance values exhibited an SD of 0.051 and an SE of 0.021, confirming that measurement variability was low relative to the magnitude of the mean.

Establishing these baseline values allowed subsequent quantification of the extent to which each bacterial treatment altered host glucose metabolism relative to untreated conditions.

Absorbance (AU) After 72 Hours of Bacterial Colonization

Spectrophotometric absorbance at 570 nm showed clear, treatment-dependent decreases following 72 hours of colonization. The untreated negative control group exhibited the highest mean absorbance (1.237 ± 0.051 AU), consistent with maintenance of baseline glucose levels. In contrast, the positive control (*E. coli* alone) demonstrated a reduction to 1.047 ± 0.050 AU, indicating that colonization with *E. coli* was sufficient to induce measurable glucose consumption within the host gut environment.

Absorbance values declined further in all four single-strain experimental treatments. *M. luteus* co-colonization (Test #1) produced a mean absorbance of 0.953 ± 0.038 AU, *B. megaterium* (Test #2) produced 0.900 ± 0.026 AU, *V. natriegens* (Test #3) produced 0.867 ± 0.022 AU, and *S. griseus* (Test #4) resulted in 0.920 ± 0.024 AU. These reductions indicated progressively stronger metabolic effects compared to the positive control. The lowest absorbance was observed in the five-strain consortium group (Test #5), which showed a dramatic decrease to 0.598 ± 0.023 AU, nearly half the value observed in the *E. coli* single-strain colonization and well below all other treatment groups. These treatment-dependent differences in absorbance are visualized in Figure 1, which shows the mean AU values with associated standard error for all eight groups and highlights the pronounced decrease observed in the multi-strain consortium.

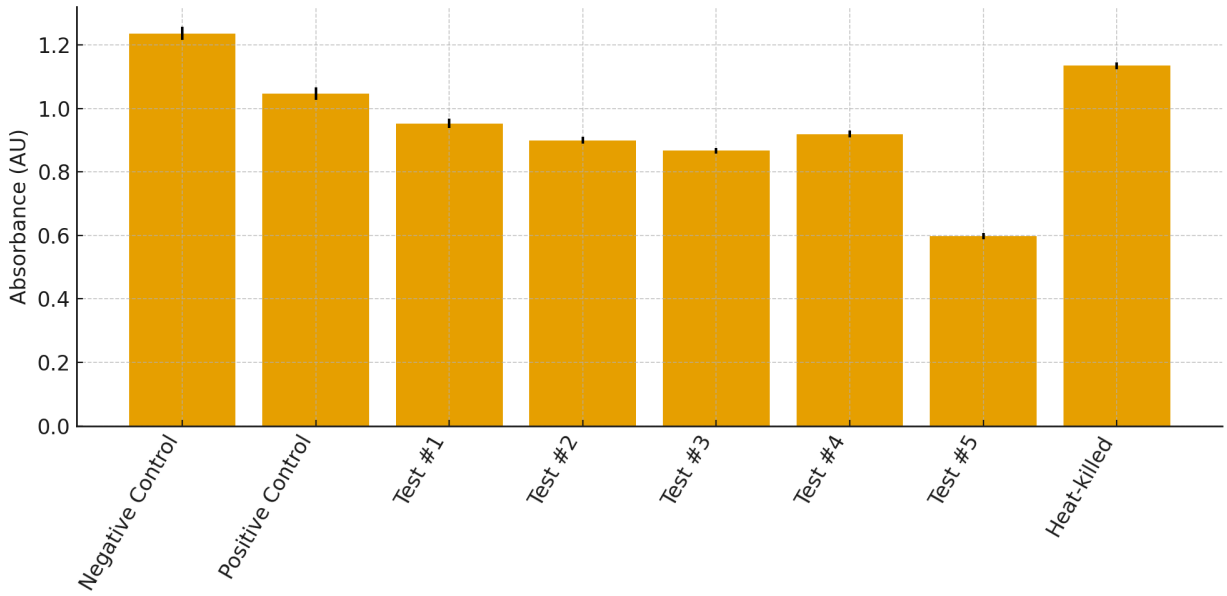

Figure 1. Mean absorbance values (AU at 570 nm) from *T. molitor* gut extracts following exposure to eight bacterial treatments (n = 6 worms per group). Error bars indicate standard error (SE). Lower AU reflects reduced glucose content.

The heat-killed *E. coli* control group exhibited a mild decrease (1.135 ± 0.027 AU), which fell between the untreated negative control and positive control. Because heat-killed bacteria cannot replicate or metabolize glucose, this slight reduction likely reflects physiological stress or minor nonspecific host responses rather than microbial metabolism. Overall, the absorbance dataset demonstrates a pronounced, graded decline in glucose-related absorbance corresponding to increasing metabolic activity of live bacterial treatments.

Glucose Concentrations Derived from the Standard Curve

Conversion of absorbance values into glucose concentrations revealed treatment effects consistent with the patterns observed in the raw AU data. The untreated negative control maintained the highest glucose concentration, averaging 274.8 ± 11.3 µM. The positive control (*E. coli* alone) showed a substantial decrease to 232.6 ± 11.0 µM, representing approximately a 20% reduction relative to uncolonized controls.

Each single-strain treatment further lowered glucose concentration. *M. luteus* colonization resulted in 211.9 ± 8.4 µM, *B. megaterium* in 200.0 ± 5.8 µM, *V. natriegens* in 192.6 ± 4.8 µM, and *S. griseus* in 204.4 ± 5.3 µM. These concentrations correspond to reductions of approximately 25-32% from the untreated condition. The greatest effect was again observed in the five-strain consortium, which exhibited a mean glucose concentration of 133.0 ± 5.1 µM. The distribution of glucose concentrations for each treatment group is shown in Figure 2, which mirrors the pattern observed in the absorbance data and illustrates the steep decline in glucose levels produced by the multi-strain consortium.

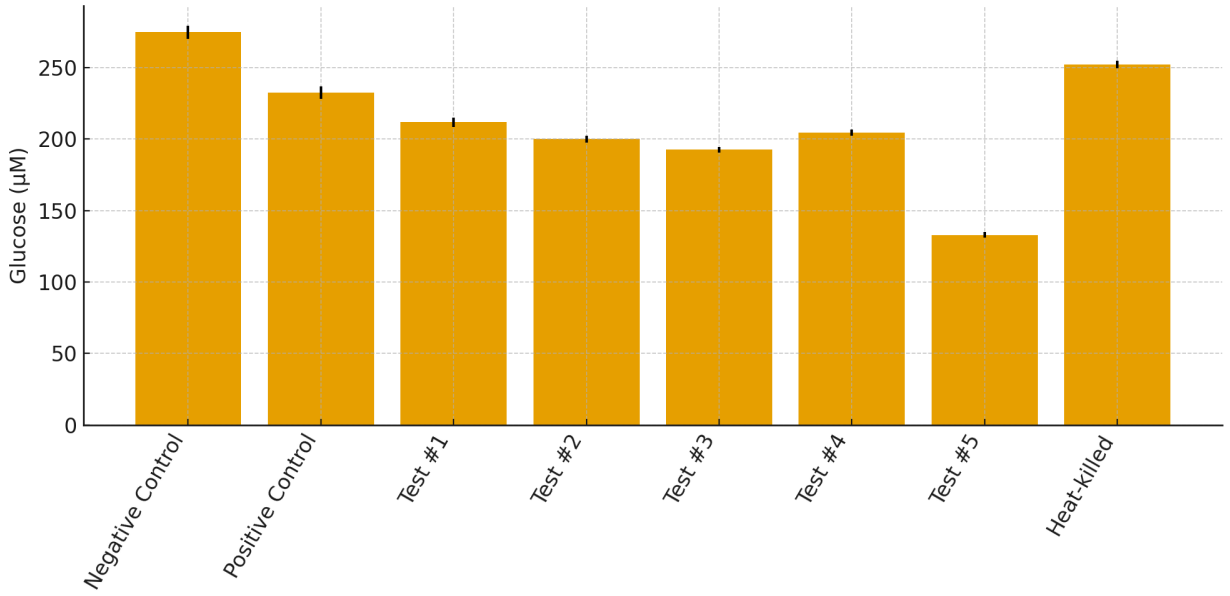

Figure 2. Mean glucose concentrations (µM) measured using the GOD–POD assay in worms exposed to each bacterial treatment (n = 6). Error bars show standard error (SE). The multi-strain consortium displays the lowest glucose levels.

This represents a reduction of about 53%, nearly double the magnitude induced by any single-strain treatment. The heat-killed *E. coli* control retained moderately high glucose levels (252.2 ± 6.1 µM), only modestly lower than the untreated group. This confirms that live bacterial metabolism—not the mere presence of bacterial biomass—is required to elicit significant glucose depletion. These findings illustrate a clear dose-like relationship between the metabolic capacity of each treatment and the extent of glucose reduction observed in *T. molitor*.

Percent Reduction in Glucose Relative to Baseline

Percent reduction calculations provided a unified metric for comparing metabolic effects across treatments. Relative to baseline negative control values, *E. coli* alone reduced glucose by approximately 20%. The four single-strain treatments produced progressively greater reductions, ranging from 25% to 32%, consistent with their lower absorbance and glucose values. Notably, the five-strain consortium produced a marked 53% reduction, emphasizing the enhanced metabolic activity of multi-strain interactions. These differences in magnitude are summarized in Figure 3, which directly compares the percent reduction in glucose relative to the negative control and emphasizes the disproportionately strong effect of the multi-strain consortium.

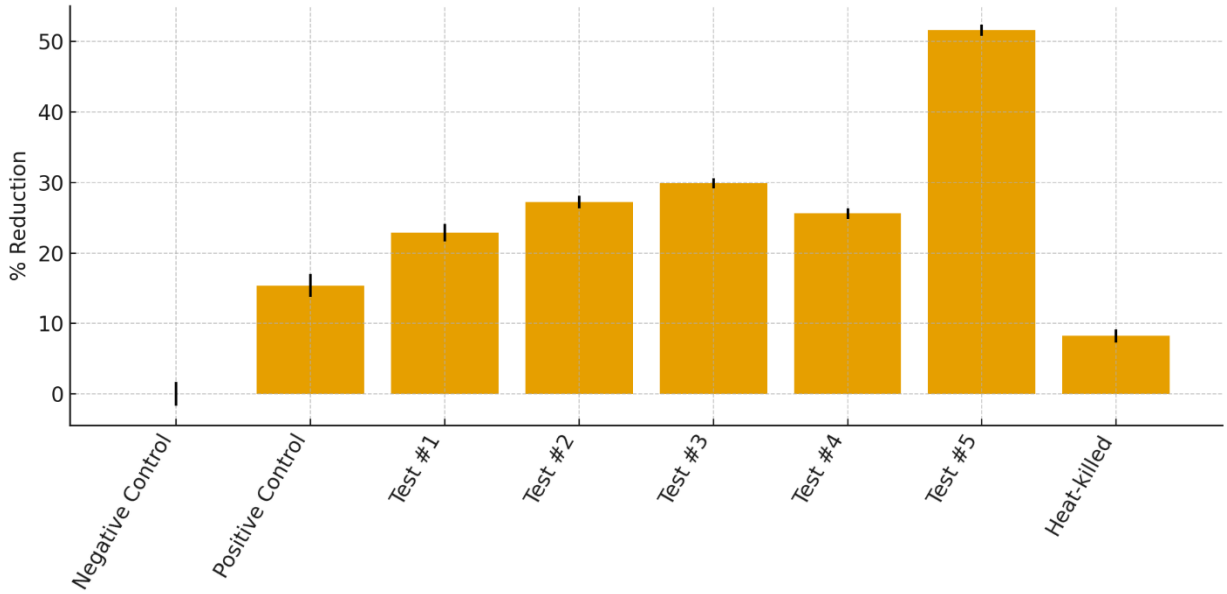

Figure 3. Percentage decrease in glucose concentration for each bacterial treatment compared with untreated controls after 72 hours. Error bars represent propagated standard error (SE). The five-strain consortium shows the greatest reduction.

Heat-killed *E. coli* produced only a 12% reduction, further supporting the conclusion that live bacterial activity is required to drive measurable glucose depletion.
The percent reduction analysis aligns closely with both absorbance and calculated glucose concentrations, reinforcing the internal consistency of the dataset and highlighting strong differentiation between treatment groups.

One-Way ANOVA for Group Comparisons

To statistically evaluate differences in glucose concentration across the eight treatment groups, a one-way ANOVA was performed. The model revealed a highly significant effect of treatment on glucose levels.

$$F(7,40) = 187.742, p = 1.578 \times 10^{-28}$$

The ANOVA results are summarized in Table 2, showing a highly significant overall effect of treatment group on glucose levels.

Table 2. One-way ANOVA of glucose concentrations (μM) across all eight treatment groups. There was a significant effect of treatment on gut glucose levels ($F_{7,40}$ = 187.74, p < 0.0001), indicating that bacterial colonization type strongly influences glucose metabolism.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-value	p-value
Treatment (Group)	7	76,850.62	10,978.66	187.74	< 0.0001
Residual (Error)	40	2,339.10	58.48	—	—
Total	47	—	—	—	—

The extremely small p-value confirms that glucose concentrations differ substantially across groups beyond what can be attributed to random variation. The magnitude of the F-statistic suggests that the between-group differences account for nearly all variability in the dataset, further supporting the strong treatment-dependent effects observed in the descriptive analyses.

Post-Hoc Pairwise Comparisons (Tukey HSD)

Tukey's Honestly Significant Difference (HSD) test identified specific pairwise differences among all treatments while controlling for familywise error. Nearly all comparisons involving the five-strain consortium were highly significant, with adjusted p-values < 0.001. This indicates that the consortium reduced glucose concentrations to a significantly greater extent than all single-strain treatments, the positive control, and both negative controls.

Comparisons between the untreated negative control and each single-strain treatment were also significant at the p < 0.001 level, confirming that colonization with any live bacterial strain meaningfully alters host glucose levels. Some comparisons among single-strain treatments, particularly between *M. luteus*, *B. megaterium*, and *S. griseus*, did not reach significance, reflecting their similar metabolic profiles. However, *V. natriegens* displayed significantly stronger effects than several of the other single-strain treatments, consistent with its lower mean glucose levels. These pairwise statistical relationships are further illustrated in Figure 4, where the Tukey HSD significance matrix provides a visual overview of which treatment pairs differ significantly in glucose concentration.

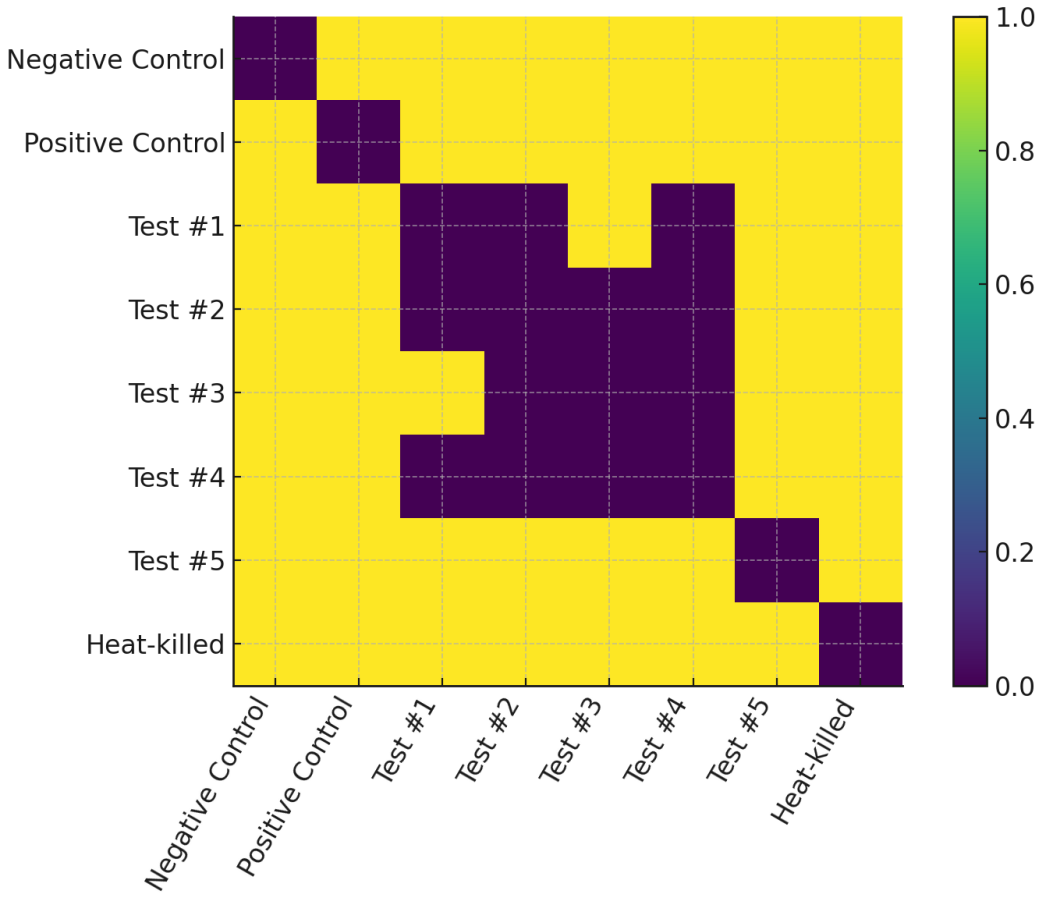

Figure 4. Heatmap summarizing significant (1) and nonsignificant (0) pairwise differences in glucose levels among all treatment groups based on Tukey's HSD test (α = 0.001).

The heat-killed *E. coli* group differed significantly from most live bacterial treatments but did not differ significantly from the untreated negative control, reinforcing its role as a non-metabolically active control. Together, the Tukey HSD results provide robust statistical confirmation that treatment-dependent reductions in glucose are driven by the metabolic activity of live bacterial species, with the multi-strain consortium exhibiting the strongest effect.

Summary of Treatment Effects

Across all quantitative metrics—absorbance, calculated glucose concentration, percent reduction, ANOVA, and Tukey HSD—bacterial colonization demonstrated a strong and statistically supported influence on host glucose metabolism. The multi-strain treatment consistently produced the greatest reduction in glucose availability, followed by the single-strain treatments, the positive control, and the two negative controls. The consistency across multiple analytical approaches reinforces the reliability of the dataset and demonstrates a clear hierarchy of metabolic impact among the bacterial treatments.

Discussion

This study demonstrates that colonization of *Tenebrio molitor* with defined bacterial strains significantly alters host glucose metabolism, with the strongest effect produced by a five-strain consortium. Whereas *E. coli* alone produced a moderate glucose reduction, all four marine sponge–analog strains caused deeper decreases, and the multi-strain community produced a notably synergistic 53% reduction relative to uncolonized controls. The heat-killed *E. coli* control exhibited only minimal reductions, confirming that live microbial metabolism drives the observed effects. Together, these findings support the emerging concept that microbial community composition strongly influences host metabolic homeostasis.

Mechanistic Interpretation of Metabolic Effects

Live gut bacteria are known to influence host metabolism through carbohydrate fermentation, glycolytic activity, and production of metabolites such as short-chain fatty acids (SCFAs), organic acids, and signaling molecules [9,10]. In insects, gut microbes contribute to nutrient assimilation, energy balance, and carbohydrate processing, often complementing host digestive enzymes [11,12]. The marked glucose reduction observed in this study is consistent with direct bacterial utilization of luminal glucose or microbial transformation of dietary sugars into metabolic end-products such as acetate or lactate.

The minimal effect observed in the heat-killed controls further supports the conclusion that metabolic activity—rather than immune stimulation or nutrient sequestration—is responsible for glucose depletion. Many of the strains used (e.g., *Bacillus megaterium*, *Vibrio natriegens*, *Streptomyces griseus*) possess enzymatic repertoires optimized for carbohydrate breakdown and energy metabolism [6,8]. This likely explains why single-strain colonization surpassed the metabolic impact of *E. coli* alone.

Synergistic Activity in the Multi-Strain Consortium

The five-strain bacterial consortium consistently produced a glucose reduction far exceeding any individual strain, indicating a synergistic community effect. Synergy in mixed microbial communities is well documented: taxa frequently perform complementary metabolic reactions, partition metabolic niches, and engage in cross-feeding, where metabolic byproducts of one species fuel the metabolism of another [13,14]. This type of functional cooperation has been shown to enhance carbohydrate catabolism and accelerate substrate turnover in both mammalian and insect gut systems [15].

Marine sponge microbiomes—on which our bacterial selections were based—are particularly known for metabolic complementarity and cooperative biosynthesis [5]. The strong effect of the five-strain treatment suggests that even a simplified consortium can recreate aspects of these naturally synergistic systems, magnifying metabolic influence on the host.

Comparison With Existing Microbiome Literature

Our findings align with previous reports showing that the gut microbiota can modulate host glucose homeostasis in mammals and insects. In humans, gut microbiome composition is increasingly recognized as a determinant of glycemic variability, insulin sensitivity, and postprandial glucose responses [16,17]. In insects, microbial colonization has been linked to changes in carbohydrate metabolism, nutrient acquisition efficiency, and host growth [18,19]. However, few studies have provided direct biochemical measurements of glucose depletion in insect gut systems following controlled bacterial colonization. Therefore, this study adds quantitative, mechanistic insight to a field that often relies on indirect metabolic readouts such as body mass or developmental timing.

Broader Implications for Metabolic Microbiome Research

This work highlights *T. molitor* as a tractable, reproducible model for studying host–microbiome metabolic interactions. Its compartmentalized gut, manageable microbiota, and ease of generating controlled colonization conditions make it suitable for mechanistic metabolic studies. Furthermore, the strength of the multi-strain synergy observed suggests that engineered microbial consortia could serve as powerful tools for modulating host metabolic profiles—a concept of growing interest in probiotic development, metabolic engineering, and microbiome therapeutics [20,21].

Because sponge-derived bacterial taxa have previously been studied for bioactive metabolite production and metabolic specialization, they represent promising candidates for exploring novel host–microbe metabolic interactions.

Methodological Strengths

Multiple features of the experimental design strengthen confidence in the findings: parallel measurements of raw (AU) and converted (μM) glucose values; uniform replication (n = 6 per group); careful use of sterile technique; use of heat-killed controls to distinguish metabolic versus structural effects; and robust statistical analyses (ANOVA + Tukey HSD) demonstrating strong treatment effects. These choices maximize reliability and reduce confounding factors.

Limitations

Several limitations must be acknowledged. First, glucose measurements were performed at a single 72-hour endpoint, preventing analysis of temporal metabolic dynamics. Second, we did not quantify the bacterial load or colonization efficiency of each strain, leaving uncertainty about whether glucose differences reflect metabolic activity, differential colonization, or both. Third, our biochemical analysis focused solely on glucose; additional metabolites such as SCFAs, amino acids, lactate, or trehalose could reveal more nuanced effects on host metabolism. Finally,

synergy was inferred from metabolic outcomes rather than directly measured using transcriptomics, metabolomics, or microbial interaction assays.

Future Directions

Future studies should incorporate multi-timepoint sampling to characterize glucose dynamics, quantify bacterial abundance using CFU counts or qPCR, and perform metabolomic profiling of gut contents. Testing intermediate consortia (pairs, triplets) would help disentangle which bacterial interactions drive synergy. Additionally, assessing host physiological responses—gene expression, energy stores, immune markers—would clarify how metabolic changes influence host biology. Extending this work to mammalian cell systems or vertebrate models could explore whether sponge-derived consortia modulate glucose metabolism across taxa.

Overall, our findings provide evidence that both individual bacterial strains and multi-strain communities significantly modulate glucose metabolism in *T. molitor*, with the strongest effects arising from synergistic activity in a metabolically diverse consortium. This study establishes a foundation for using simplified insect gut models to examine how microbial communities influence host metabolic homeostasis and identifies sponge-inspired bacterial assemblages as promising tools for future microbiome engineering efforts.

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