A Genetically Modified Anti-*Plasmodium* Bacterium Is Harmless to the Stingless Bee *Partamona Helleri*

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Research Article

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Abstract

Paratransgenesis consists of genetically engineering an insect symbiont to control vector-borne diseases. Biosafety assessments are a prerequisite for the use of genetically modified organisms (GMOs). Assessments rely on the measurement of the possible impacts of GMOs on different organisms, including beneficial organisms, such as pollinators. The bacterium *Serratia* AS1 has been genetically modified to express anti-*Plasmodium* effector proteins and does not impose a fitness cost on mosquitoes that carry it. In the present study, we assessed the impact of this bacterium on the native bee *Partamona helleri* (Meliponini), an ecologically important species in Brazil. *Serratia* eGFP AS1 (recombinant strain) or a wild strain of *Serratia marcescens* were suspended in a sucrose solution and fed to bees, followed by measurements of survival, feeding rate, and behavior (walking and flying). These bacteria did not change any of the variables measured at 24, 72, and 144 h after ingestion. Recombinant and wild bacteria were detected in the digestive tract up to 144 h after ingestion, but their numbers decreased with time. The recombinant strain was detected in the midgut at 24 h and in the hindgut at 72 h and 144 h after ingestion. As reported for mosquitoes, *Serratia* eGFP AS1 is a safe candidate for combating vector-borne diseases, as it did not compromise the native and ecologically relevant bee, *P. helleri*.

1. Introduction

Malaria is one of the deadliest infectious diseases worldwide. *Plasmodium* parasites, the causative agents of malaria, are transmitted to humans through the bite of infected *female Anopheles* mosquitoes. Control of malaria is based primarily on reducing vector populations with insecticides and using antimalarial drugs [1]. These tools have been ineffective due to the development of mosquito insecticide resistance and parasite drug resistance [1]. The development of new tools to combat this disease is of high priority.

Paratransgenesis, the genetic manipulation of insect symbiotic microorganisms to block pathogen transmission, is a promising strategy for controlling insect-borne diseases. Its effectiveness is enhanced by the fact that bacteria share the same compartment, the midgut, with the pathogens transmitted by the insects and because bacterial numbers increase dramatically following a blood meal [2, 3]. The facultative aerobic and gram-negative rod-shaped bacteria of the genus *Serratia* (Enterobacteriaceae) are common components of the midgut microbiota. This genus is a symbiont of many arthropods, such as mosquitoes, bees, sandflies, ticks, and aphids [4–9].

The potential of *Serratia* eGFP AS1 (*Serratia marscescens*, AS1 strain) for paratransgenesis has been demonstrated [10–12]. The gene encoding green fluorescent protein (eGFP) has been integrated into the *Serratia* eGFP AS1 chromosome. This bacterium contains a plasmid with five anti-*Plasmodium* effector genes [(MP2) 2 - Scorpine - (EPIP) 4 - Shiva1 - (SM1) 2] under the control of a single promoter, which inhibits the development of *Plasmodium falciparum* in female *Anopheles gambiae* and *Anopheles stephensi* [10]. Both SM1 and MP2 (midgut peptides 1 and 2, respectively) bind to the mosquito midgut surface and inhibit *Plasmodium* invasion [13, 14]. Scorpine is an antimicrobial peptide found in the venom of the scorpion *Pandinus imperator* and prevents the formation of gametes and ookinetes of *Plasmodium berghei* [15]. EPIP (enolase-plasminogen interaction peptide) inhibits mosquito midgut invasion by preventing plasminogen binding to the ookinete surface [13]. The Shiva1 or cecropin-like synthetic antimicrobial lytic peptide kills *P. falciparum* [16]. All these effector proteins strongly inhibited this pathogen, reducing the oocyst load by up to 93% [10].

The use of any genetically modified organism (GMO) for biological control should impose minimal fitness cost to its insect carrier [2]. Moreover, a thorough risk assessment to the environment is required prior to introduction in the field [17]. These assessments include investigating the transfer routes of GMOs, which can be vertical (from mother to offspring), transstadial (between developmental stages), and horizontal (from one individual to another, without being parental), as well as from the effects of the GMO on behavior, survival, and reproduction of potential hosts [10, 11].

The horizontal transfer of the GMO between organisms can occur through the sharing of common resources [10, 18, 19], for example, by water contaminated with the GMO [12]. Normative institutions, such as CTNBio (National Technical Commission of Biosafety, Ministry of Science, Technology and Innovation, Brazil), also require the assessment of the interaction of paratransgenic individuals with the environment, which includes a particular concern regarding the possible harmful effects of GMO on non-target organisms [17], including pollinators [20]. Since bees, as pollinators, play a significant role in maintaining biodiversity [21, 22], these organisms are widely recognized for integrating risk assessment protocols [23–26].

Forager bees perform out-colony tasks, including the search for food resources (i.e., water, fiber, resin, nectar, and pollen), which are in direct contact with the external environment [27]. Therefore, risk assessments are preferably carried out on foragers and include assessments of lethality and, to a lesser extent, sublethal effects [28, 29]. Such assessment studies are mostly carried out with the honey bee *Apis mellifera* as a model organism, but this species is exotic in Neotropical environments such as South America [29, 30]. In this sense, stingless bees (Meliponini) are more representative of Neotropical ecosystems [22, 29] as pollinators of native and cultivated plants [31]. Therefore, stingless bees should be considered in studies to measure the potential risks of GMOs.

The ability of bees to withstand environmental stressors is linked to the gut microbiota [32, 33]. In addition, the gut microbiota can influence the behavior, metabolism, growth, and development of hosts [34, 35]. The microbiota is highly conserved among several species of stingless bees [36]. The stingless bee *Partamona helleri* (Meliponini) has a wide range of dominant bacterial genera (approximately 33), and the genus *Serratia* has also been found in this species [8]. Certain *Serratia* species can also be pathogenic to bees, as was observed for the *S. marcescens sicaria* strain (Ss1) in honeybee adults [37].

This work evaluated the risk to adult *P. helleri* workers of ingestion of the genetically modified *Serratia* eGFP AS1, carrying anti-*Plasmodium* effectors. We investigated survival, food ingestion (i.e., feeding rate), and walking and flight activities of adults as it relates to the evaluation of safety to the environment.

2. Materials And Methods

2.1. Bees

Foragers from five colonies of *P. helleri*, 25 or more days old [27] were obtained from the Central Apiary at the Universidade Federal de Viçosa (UFV), Viçosa - MG (20º 45 '14" 'S; 42º 52' 55") under license ID75536 (ICMBio – SISBIO, Ministério do Meio Ambiente, Brazil). The foragers were captured with a glass bottle at the hive entrance and immediately transported to the Insect Molecular Biology Laboratory (UFV) without controlling the temperature or luminosity. The bees were anesthetized with carbon dioxide for 5 s and then transferred to 500 mL round transparent plastic pots. The foraging bees fasted for 1 h in an incubator (28 ± 1°C, 70 ± 5% relative humidity (RH), in the dark) until the bioassays began. This fasting period is necessary to stimulate the ingestion of food provided in oral exposure tests [38].

2.2. Bacteria

Serratia eGFP AS1 (GenBank: KY935421) was genetically modified to express eGFP (pBAM2-GFP), antimalarial effector proteins [(MP2) 2 - Scorpine - (EPIP) 4 - Shiva1 - (SM1) 2)], and resistance to the antibiotic kanamycin. This strain has a yellowish beige color [10]. The wild strain *S. marcescens* (wild strain MIND01) synthesizes prodigiosin, a naturally reddish pigment at 28°C, and does not grow in the presence of kanamycin. The recombinant bacterium was provided by the Department of Molecular Microbiology and Immunology, Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health (Baltimore, USA). The wild bacterium was kindly provided by Dr. Maria Cristina Dantas Vanetti at the Industrial Microbiology Laboratory (UFV). *Serratia* eGFP AS1 was cultured under license CQB 024/97 at the Laboratório de Imunoquímica e Glicobiologia (DBG-UFV). Both strains were grown for 24 h at 28°C in Petri dishes containing Luria Bertani (LB) broth (composition per liter: 10 g tryptone, 5 g yeast extract, and 10 g NaCl) (Lenoxx - L3022 Sigma-Aldrich, Saint Louis, Missouri, USA), with 2% agar (Himedia ® - RM026 Technical Data, Mumbai, Maharashtra, India), supplemented with or without kanamycin sulfate (100 µg/mL) (Gibco™ – 11815032 ThermoFisher, Burlington, Ontario, Canada) to obtain isolated colonies. Subsequently, bacteria from each isolated colony were cultured separately for 24 h at 28°C in 100 mL glass test tubes containing 5 mL of liquid LB medium. Bacteria stocks in LB medium supplemented with 20% glycerol were stored at -80°C for later use.

2.3. Exposure to the bacteria

The bacteria (recombinant and wild) stocks were thawed and cultured for 24 h in liquid LB medium at 28°C and diluted in autoclaved sucrose solution (50% v/v) to a final concentration of 10^8 cells/mL, which was verified with the final optical density (OD) 600 nm of 0.1. Different sucrose solutions were prepared to expose the bees to four separate treatments: 1) sugar only (control), 2) sterile LB with sucrose (1:1), 3) *Serratia* eGFP AS1 (recombinant) with sucrose, and 4) wild *S. marcescens* with sucrose. A total of 100 bees per treatment (20 bees from the same colony in a plastic pot × 5 colonies or replicates = 100 bees/treatment; n = 400 bees). Two independent experiments were conducted. The first assessed survival and food consumption over 144 h (n = 400 bees). The second assessed the behavior at 72 h and tested for the presence of bacteria in the digestive tract (n = 400 bees) at 24, 72, and 144 h.

Each sucrose solution was offered to 20 bees in a 2 mL microtube drilled at the bottom and inserted through a hole in a 500 mL plastic pot [38]. After 24 h exposure, the sucrose solution was changed, and the bees received 50% sterile sucrose solution (v/v) for the next 120 h.

2.4. Survival and food consumption

Survival was monitored every 24 h for 144 h. Individuals were considered dead when they did not move after stimulation with forceps, and dead bees were discarded [38].

Food consumption was measured by weighing the microtubes on an analytical scale. The weights of the microtubes with the sucrose solution were recorded before feeding and then again before switching. The food, which contained the four different diets, was offered for 24 h. The tubes with the remaining food were then weighed. After 24 h, the microtubes were replaced with microtubes containing sterile food, and the weights of the microtubes were measured 48, 72, 96, 120, and 144 h after the beginning of the assay. Plastic containers without bees, but with

microtubes containing sucrose (50% v/v), were kept under experimental conditions to estimate the losses by evaporation. These values have been used to correct food consumption [8, 38, 39].

2.5. Detection of bacteria in the digestive tract and ovary

The presence of *Serratia* eGFP AS1 and *S. marcescens* (wild) in the digestive tract of bees was assessed. Fifteen bees (3 bees per colony, totaling 5 colonies) were used for each treatment (i.e., different foods) for 24, 72, and 144 h (n = 180 bees). Anesthetized bees were kept on ice, sterilized for three min with 70% ethanol, and washed three times with sterile phosphate buffered saline (PBS; 0.1M, pH 7.2). The digestive tract (i.e., midgut and hindgut) was dissected, transferred separately to a microtube (1.5 mL), homogenized in 500 µL of sterile PBS, and serially diluted from 10^0 to 10^4. From this homogenate, the micro-drop plating technique was completed using a 20 µL aliquot, which was plated in triplicate, collected for each sample, and transferred to plates with agar and LB medium containing 100 µg/mL kanamycin [40]. The plates were then incubated at 28°C for 24 h. The bacteria were identified according to their colony phenotype (Supp. Figure 1, 2). Colony GFP fluorescence was detected using a UV transilluminator (High-Performance 2UVTM Transilluminator, λ = 365 nm).

The second set of bees from the four experimental groups was anesthetized and dissected in PBS. The digestive tract and ovaries, which are atrophied in workers, were transferred to glass slides mounted with PBS. The presence of *Serratia* eGFP AS1 was checked optically in freshly dissected organs at different time points (i.e., 24, 72, and 144 h). Samples were analyzed with an Olympus BX60 Epifluorescence Microscope coupled with a QColor Olympus® image capture system using WB filters (450–480 nm) for eGFP.

2.6. Walking

Walking activity was studied in groups of 5 individuals from the same colony inside arenas (Petri dishes, 9 cm diameter, 2 cm high). The bottom of the arenas was covered with white filter paper (ash content < 0.1%, diameter 9 cm, thickness 0.13–0.17 mm), and the upper part of the arenas was wrapped with transparent PVC film. The activities were recorded for 10 min with a digital camcorder (HDR-XR520V, Sony Corporation, Tokyo, Japan) at 30 fps and high definition (1920 × 1080 pixels). The videos (Supp. Videos) were analyzed using the Ethoflow® software (National Institute of Industrial Property-INPI, Ministry of Economy, Brazil, BR 51 2020 000737-6) [41], considering the time at resting (s) (walked distance ≤ 0.0226 cm/frame), the medium activity time (s) (0.0226 < walked distance ≤ 0.22 cm/frame), the fast activity time (s) (walked distance > 0.22 cm/frame), and cumulative walked distance (cm). Each treatment was studied in two groups of 5 individuals per colony of five different colonies; therefore, 50 individuals were evaluated per treatment, totaling 200 individuals. In the data analysis, the average of individuals in each arena was considered as a replicate. Recordings were performed under red light at 25 ± 2°C and 70 ± 5% RH [38].

2.7. Flight

The same foragers evaluated in the walking bioassay were evaluated using a flight bioassay. The assay consisted of releasing 5 bees at the bottom of a wooden tower (105 cm high × 35 cm long × 35 cm wide) covered laterally with the organza fabric. The assay was carried out in a dark room with a white lamp (60 W), 5 cm above the top of the tower. The bees were kept for 1 min at the base of the tower in a Petri dish for acclimatization and then released. The proportion of individuals that reached the top of the tower was counted [42]. Two groups of 5 individuals were analyzed for each treatment from the five different colonies (n = 200). The average proportions of the two groups in each colony were used for statistical analyses.

2.8. Statistical analysis

Survival data were used to obtain survival curves using Kaplan-Meier estimators and were first analyzed using the log-rank test. Subsequently, survival curves were pairwise compared using Bonferroni's method. The values of food consumption were transformed to *ln* and then subjected to the generalized least squares (GLS) model under different structures of variance-covariance, owing to repeated measures over time. The model was chosen based on parsimony, verification of residual quantile plots, and the lowest Bayesian information criterion (BIC). Back-transformed estimates were then used in graphical plots to represent consumption over time and between treatments. CFU data were rank-transformed and submitted to a linear mixed-effects model with the colony as a random effect. Subsequently, Tukey's pairwise comparisons were carried out using adjusted *P* values according to the Westfall method [43]. The variables of walking behavior (time at rest, medium activity time, fast activity time, and walked distance accumulated) were subjected to analysis of variance (ANOVA) with colonies considered as repetitions. For flight behavior data, a generalized linear model (GLM) was fitted with a quasibinomial distribution; adequate distribution for proportion data when there was overdispersion (high residual deviance), and colonies were also used as repetitions. The residues were checked in all models to verify the adequacy of the distributions. All data were analyzed using R software [44] with a significance level of 5%.

3. Results

Consumption of the different sucrose solutions did not affect the survival of the bees during the 144 h experiment ($\chi^2 = 8.7$, df = 3, $p = 0.03$) (Fig. 1). However, only pairwise contrast between wild and LB survival curves differed significantly ($p = 0.014$). Food consumption was significantly affected over the studied time points (i.e., 24, 48, 72, 96, 120, and 144 h) ($F_{1,106} = 76.95$; $p < 0.001$), with the highest ingestion occurring between 72 and 96 h (Fig. 2A). However, the amount of food consumed was not significantly different among the treatments (i.e., different food) during the 144 h period analyzed ($F_{3,106} = 0.16$; $p = 0.92$) (Fig. 2B).

Both *Serratia* eGFP AS1 and *S. marcescens* (wild) were detected in the homogenate obtained from the digestive tract (midgut + hindgut) dissected at 24, 72, and 144 h after the ingestion of the bacteria (Fig. 3, Supp. Figure 1). The bacterial colony-forming units (CFUs) were higher at 24 h than at 72 h (z = -3.57, p = 0.001) and 144 h (z = -2.21, p = 0.027), but were similar between 72 h and 144 h (z = 1.36, p = 0.17) for the recombinant strain. The CFUs were higher at 24 h than at 72 h (z = -3.65, p < 0.001) and 144 h (z = -4.40, p < 0.001), but were similar between 72 h and 144 h (z = -0.76, p = 0.45) for the wild strain. *Serratia* eGFP AS1 was detected in the midgut of bees at 24 h and in the hindgut at 72 h and 144 h after exposure (Fig. 4). However, this modified bacterium was not detected in the ovaries at any of the analyzed times (Supp. Figure 2).

There was no significant difference between treatments in any of the variables associated with walking activity (Supp. Table 1, Supp. Videos). The resting time (F3,16 = 0.2; P = 0.9; $R^2 = 0.15$) (Fig. 5A), medium activity (F3,16 = 0.9; P = 0, 5; $R^2 = 0.013$) (Fig. 5B), or fast activity (F3,16 = 0.0; P = 1.0; $R^2 = 0.18$) (Fig. 5C) were similar among treatments, and the accumulated distance walked (F3,16 = 0.3; P = 0.9; $R^2 = 0.13$) (Fig. 5D). Flight activity was also not affected by the treatments (F3,16 = 0.2; P = 0.89) (Fig. 6).

4. Discussion

The present study is the first to investigate the possible effects of digestive tract colonization of a native bee by a genetically modified bacterium (*Serratia* eGFP AS1) developed for the control of vector-borne diseases. Our results demonstrated that ingestion of the modified bacterium did not affect the fitness or behavior of *P. helleri* foragers. Bees colonized by *Serratia* eGFP AS1 had similar survival rates, ingested the same amount of food, and had similar walking and flying activities compared to individuals that ingested a non-recombinant bacterium or sterile sucrose controls. Our results corroborate the studies using mosquitoes (i.e., *Culex pipiens*, *A. gambie*, and *A. stephensi*) colonized by *Serratia* eGFP AS1, for which no negative effects on survival, feeding behavior, or fertility were detected [10, 11].

In general, the genus *Serratia* is non-pathogenic and is constitutively found in the guts of many arthropods, including mosquitoes, bees, sandflies, ticks, beetles, and aphids [4–9, 45]. In our experiments, ingestion of the two tested bacterial strains was not harmful to the bees. However, certain *S. marcescens* strains are pathogenic. For instance, strain Ss1 is pathogenic when in high abundance in honeybees [33, 37], whereas RPWL1 can be pathogenic to the beetle *Rhynchophorus ferrugineus* [46]. Conversely, a genetically modified *Serratia symbiotica* did not affect the fitness or survival of aphids, suggesting that it is an important future paratransgenic tool for the control of agricultural pests [9].

Serratia eGFP AS1 and wild *S. marscencens* were recovered from the digestive tract (midgut + hindgut) of *P. helleri* at 24, 72, and 144 h after bacterial ingestion, however there was a reduction in bacterial numbers over time. This reduction may be related to the specificity of the gut microbiota [34], as native strains easily outperform non-native strains [47]. However, how this occurs is still not well understood and needs to be further studied. Using fluorescence, *Serratia* eGFP AS1 was detected only in the midgut 24 h after ingestion, and only in the hindgut at 72 h and 144 h after ingestion. This change in the organ of colonization may be due to the protection conferred by gut bacteria naturally present in Meliponini bees [8, 36, 48].

In mosquitoes, *Serratia* eGFP AS1 persists for at least three consecutive generations and colonizes organs other than the midgut, including the ovaries and male accessory glands. In addition, this bacterium rapidly spreads among mosquito populations in the laboratory [10]. In bees, possible contamination with the bacteria occurs when they leave the colony for foraging, a function performed at the end of the bee's life [27]. Once contaminated with bacteria, foragers return to the colony where they can contaminate other individuals. However, as *Serratia* eGFP AS1 was not detected in the ovaries of the bees, it is unlikely that this bacterium could spread through bee populations via vertical transfer. This study highlights the importance of studying the effects of GMOs on non-target organisms, including pollinators, to aid in decision making for the release of GMOs into the environment.

Declarations

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Compliance with Ethical Standards

Disclosure of potential conflicts of interest

The authors declare that they have no conflict of interest.

Research involving Human Participants and/or Animals

No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

Informed consent

Not applicable

Authors' contributions

TAV, WFB, RCB, MJL, GFM conceived this research and designed experiments; TAV, LLBJ, RCB collected the data; TAV, WFB, LLBJ, RCB, JSS, GFM analysed the data; TAV and GFM wrote the manuscript. TAV, WFB, LLBJ, JSS, MJL, GFM corrected the work, contributed critically to the drafts and approved for publication.

Data Availability

The two bacterial strains used in the experiments are stored in the Laboratório de Biologia Molecular de Insetos at UFV, Brazil. The data that support the findings are available from the corresponding author upon request.

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Figures

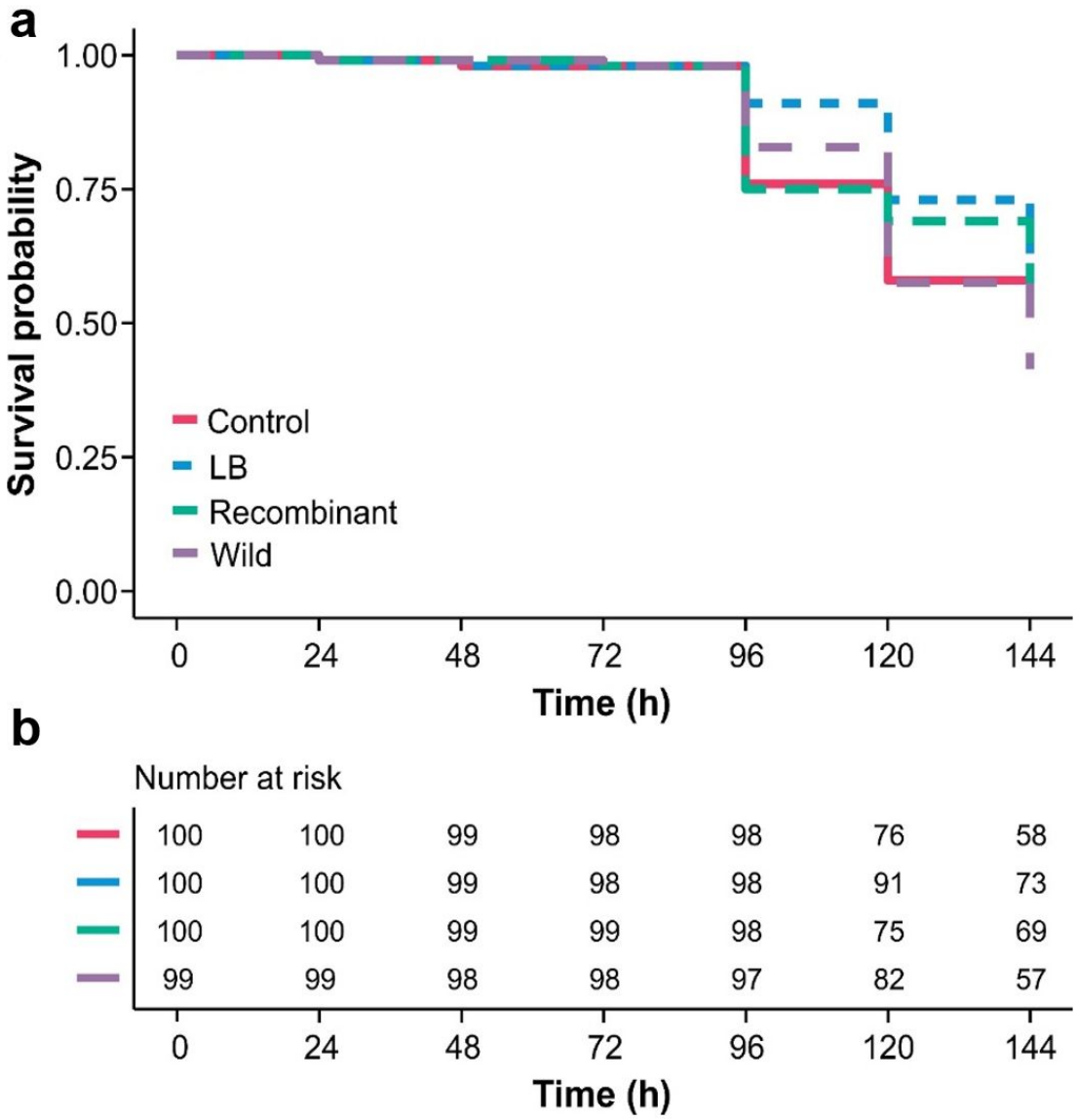

Figure 1

(A) Survival of Partamona helleri fed with sterile sucrose solution (control), sterile sucrose solution with sterile LB (1:1), sucrose solution containing Serratia eGFP AS1 (recombinant), and sucrose solution containing Serratia marcescens (wild). The overall log-Rank test indicates a significant difference in at least one of the contrasts between treatments (x2 = 8.7, df = 3, p = 0.03). (B) Number of bees remaining at risk per treatment for each assessed time

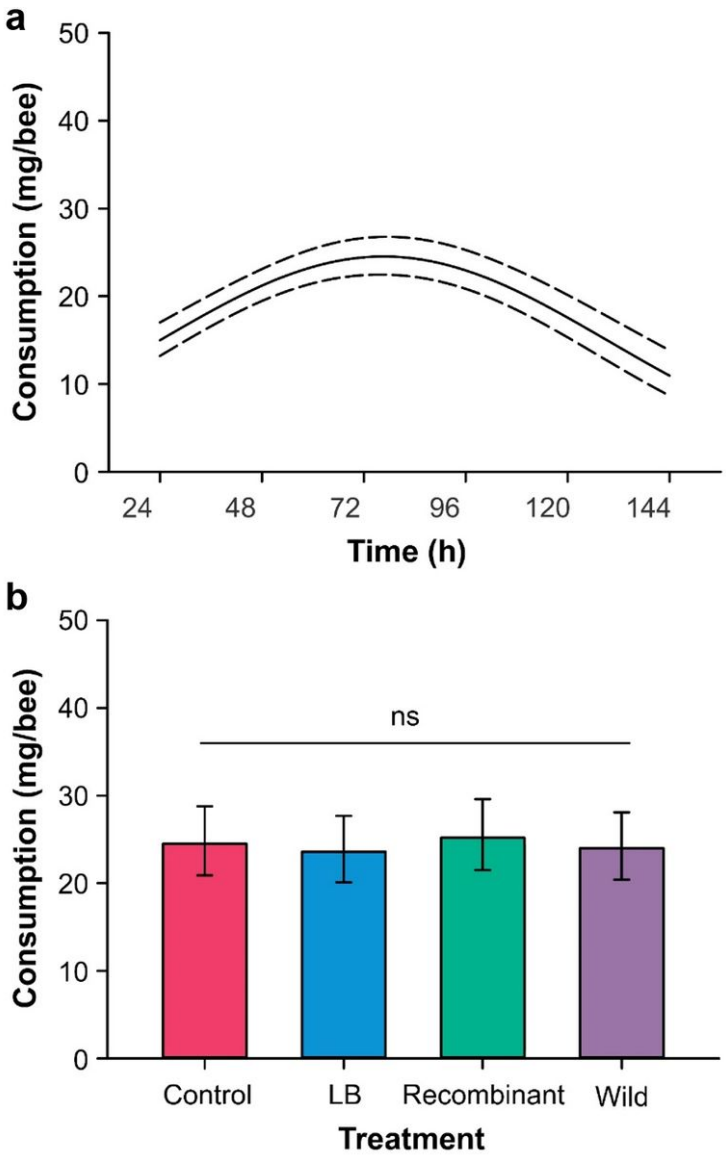

Figure 2

Ingestion of sucrose solution (mg/bee) by Partamona helleri. (A) Food consumption considering all treatments versus time after exposure. (B) Consumption according to treatments: sterile sucrose (control), sterile sucrose plus sterile LB (1:1), Serratia eGFP AS1 (recombinant) in sucrose, and Serratia marcescens (wild) in sucrose. The values are means of five biological replicates, 20 bees per treatment (n=400). ns: not significant; solid line and bars: means (± 95% confidential intervals)

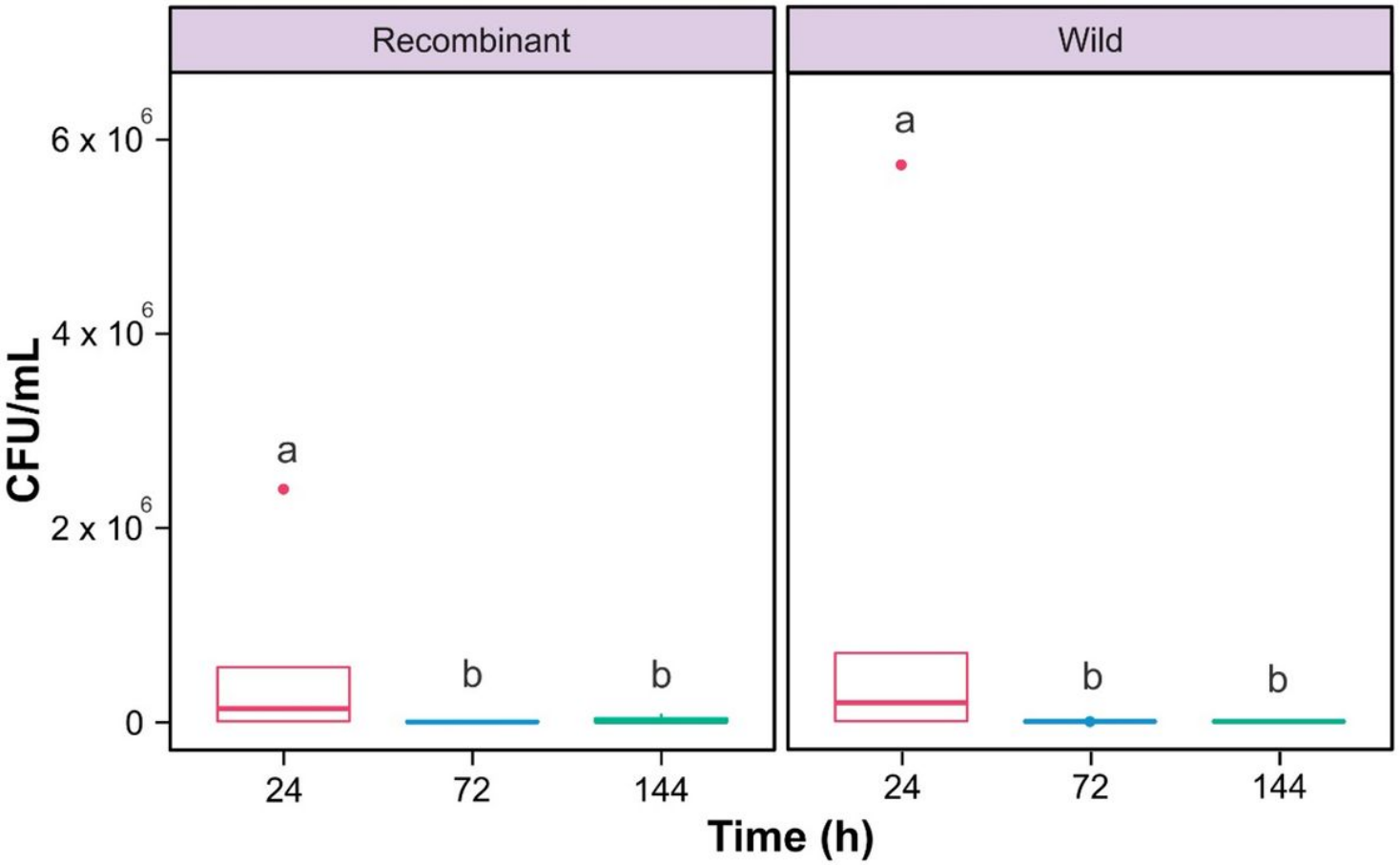

Figure 3

Colony-forming units (CFU/mL) of Serratia eGFP AS1 (recombinant) and Serratia marcescens (wild) obtained from Partamona helleri disgestive tract homogenates. CFU were higher at 24 h than at 72h ($z = -3.65$, $p < 0.001$) and 144h ($z = -4.40$, $p < 0.001$), but it was similar between 72 h and 144 h ($z = -0.76$, $p = 0.45$) for the wild strain. CFU were higher at 24 h than at 72 h ($z = -3.57$, $p = 0.001$) and 144 h ($z = -2.21$, $p = 0.027$), however, it was similar between 72 h and 144 h ($z = 1.36$, $p = 0.17$) for the recombinant strain. The CFUs are means of five biological replicates in groups of 3 bees per treatment ($n = 90$). Different letters indicate significant differences (Tukey's test; $\alpha = 5\%$)

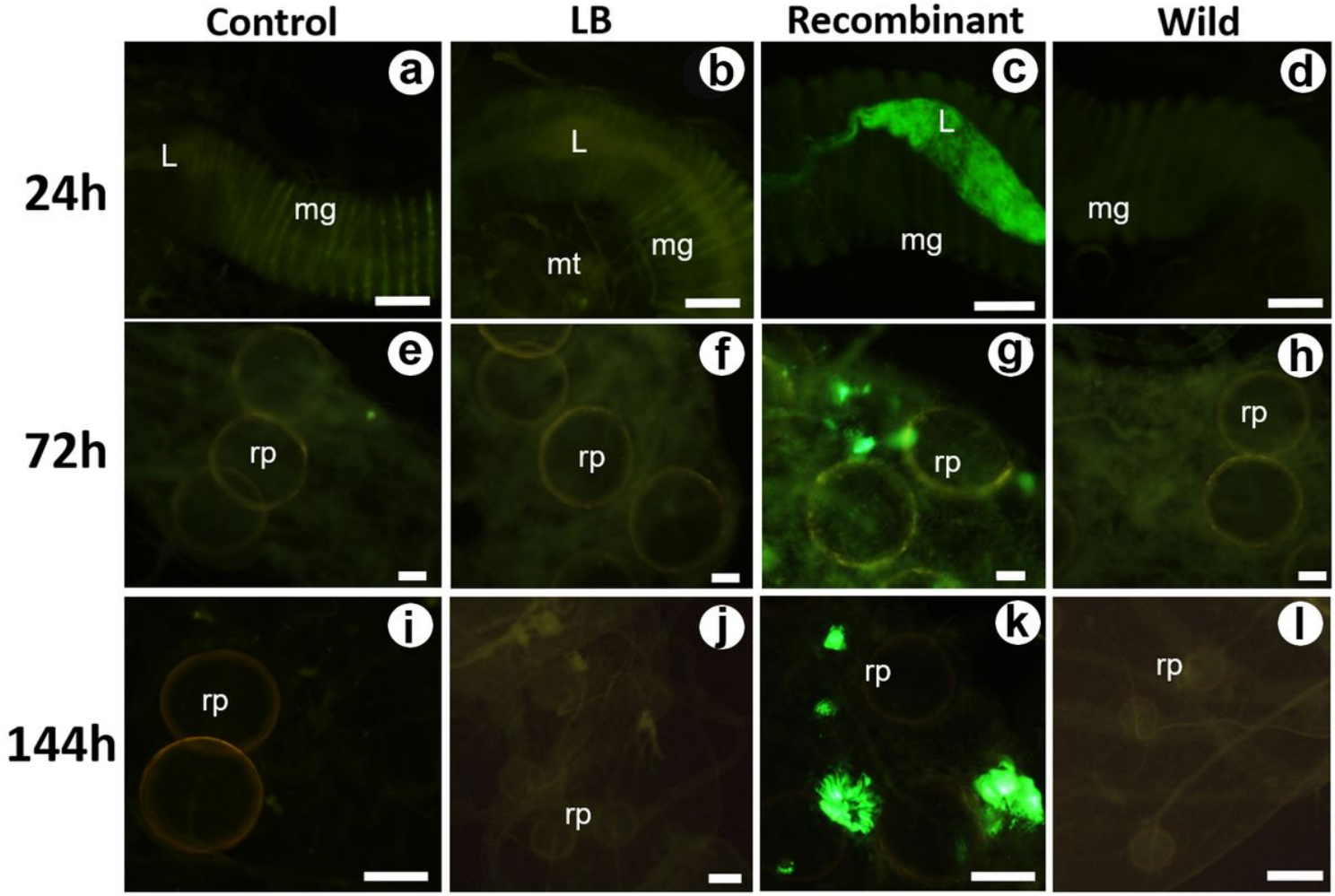

Figure 4

Midgut and hindgut of workers of Partamona helleri after the ingestion of different sucrose solutions. (A) Midgut at 24 h after the ingestion of sterile food (control). (B) Midgut at 24 h after the ingestion of food with sterile LB (1:1). (C) Midgut at 24 h after the ingestion of food with Serratia marcescens (wild). (D) Midgut at 24 h after the ingestion of food with Serratia eGFP AS1 (recombinant, green fluorescent). (E) Hindgut at 72 h after the ingestion of sterile food (control). (F) Hindgut at 72 h after the ingesting of food with sterile LB (1:1). (G) Hindgut at 72 h after the ingestion of food with S. marcescens (wild). (H) Hindgut at 72 h after the ingestion of food with Serratia eGFP AS1 (recombinant). (I) Hindgut at 144 h after the ingestion of sterile food (control). (J) Hindgut at 144 h after the ingesting of food with sterile LB (1:1). (K) Hindgut at 144 h after the ingestion of food with S. marcescens (wild). (L) Hindgut 144 h after the ingestion of food with Serratia eGFP AS1 (recombinant). Midgut lumen (L); midgut (mg); rectal pads (rp) in the hindgut. Bars for 24 h time points: 200 µm; Bars for 72 h time points: 50 µm; Bars for the 144 h time points: 100 µm

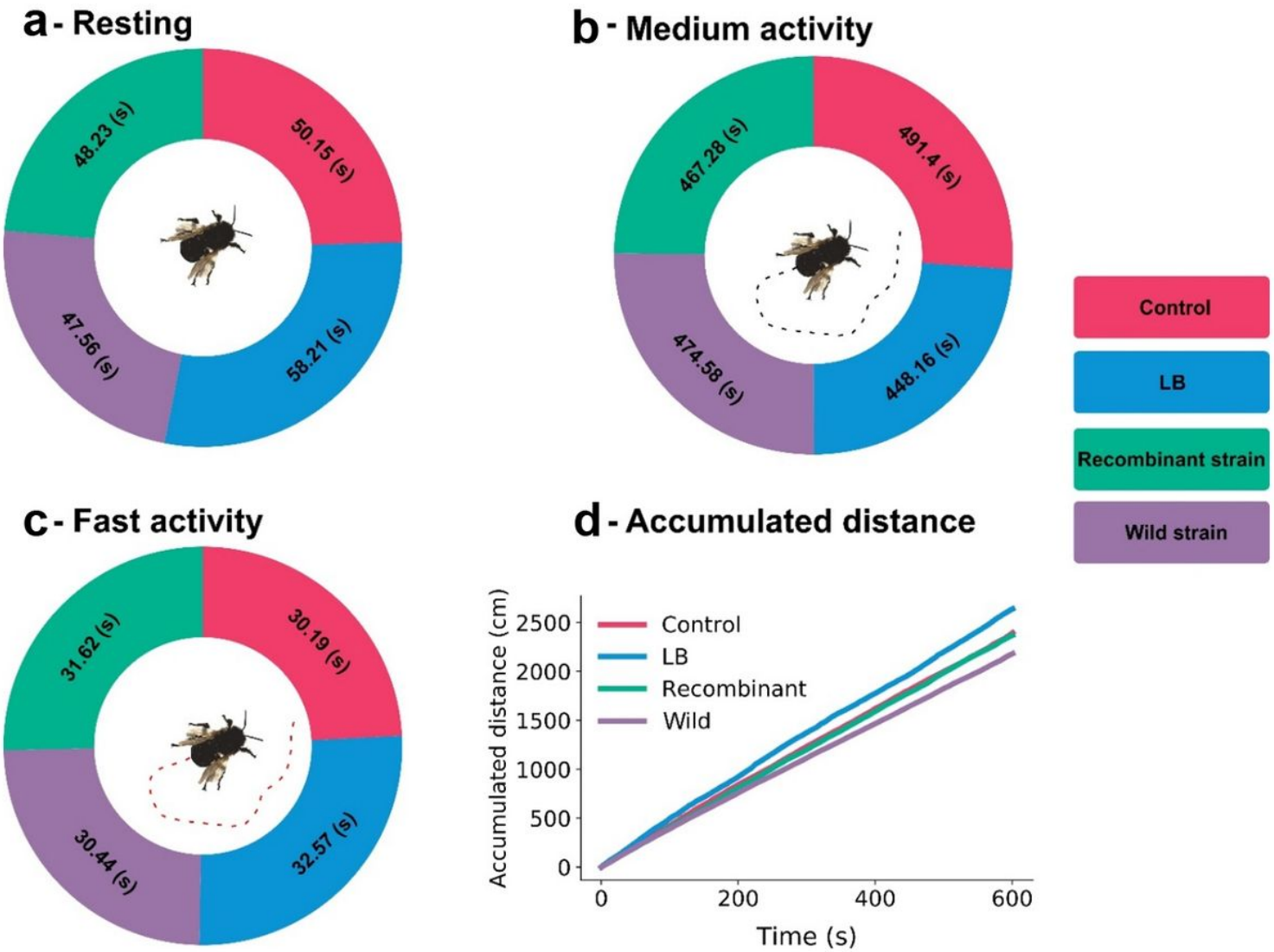

Figure 5

The walking activity of Partamona helleri after ingestion of different sucrose solutions: not inoculated (control), with sterile LB (1:1), with Serratia eGFP AS1 (recombinant), and with Serratia marcescens (wild). The constituent values within each part of the graph represent the averages obtained from (A) time at rest, (B) medium activity time, (C) fast activity time, and (D) walked distance accumulated over 10 minutes. The walking activity is the mean of five biological replicates in two groups of 5 bees per treatment (n=200). There was no significant difference in any of the variables based on the analysis of variance (α = 5%)

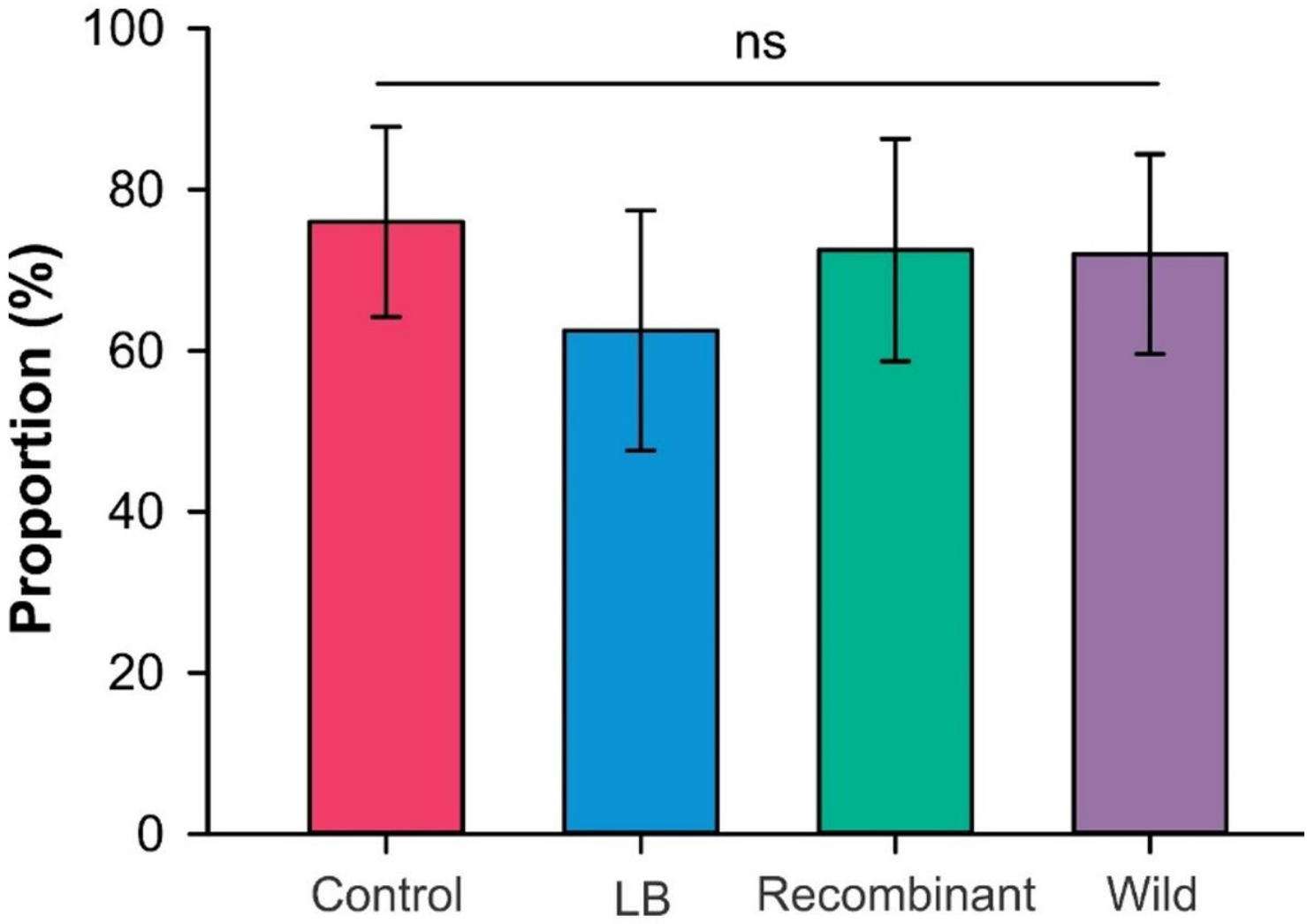

Figure 6

The proportion of foragers of Partamona helleri that flew after ingestion of different sucrose solutions: not inoculated (control), with sterile LB (1:1), with Serratia eGFP AS1 (recombinant), and with Serratia marcescens (wild). The average proportion of five biological replicates in two groups of 5 bees per treatment (n=200). There was no significant difference in any of the variables analyzed according to the generalized linear model (α = 5%), ns: indicates not significant; bars: mean (± standard error)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Sup.Material.docx
- Sup.Video1Control.mp4
- Sup.Video2LB.mp4
- Sup.Video3Transgenicstrain.mp4
- Sup.Video4Wildstrain.mp4