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Exploration of entomopathogenic bacteria as potential control agents for brown stink bug *Euschistus heros* (F.) (Hemiptera: Pentatomidae)

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ABSTRACT
The brown stink bug, *Euschistus heros*, is considered one of the main pests in Brazil, causing significant damage to several crops. Currently, the principal method of control involves the excessive use of insecticides, leading to the development of resistant populations and environmental contamination. Therefore, it becomes crucial to explore more sustainable control alternatives, with biological agents, particularly entomopathogenic bacteria, emerging as promising due to their proven toxic activity against various insect and insect families. Thus, this study aimed to explore the potential of entomopathogenic bacteria in the control of *E. heros*. The initial screening of 125 bacteria identified 19 efficient strains, which were tested at 10% concentration under laboratory conditions. Molecular identification was conducted by polymerase chain reaction amplification of the 16S, gyrB, and rpoD genes, followed by sequencing and comparison in EzBioCloud 16S and GenBank. Additionally, the survival rate of *E. heros* was evaluated at bacterial concentrations of 10, 20, 40, 80, and 100%. Among the isolates tested at 10% concentration, strains 292B3, 457C4, 365BNP6, 742D, 427B, 321B, and *Photorhabdus luminescens* emerged as the most virulent. Molecular analysis of these strains revealed high similarity to the species *Serratia marcescens*, *Bacillus toyonensis*, and *Bacillus cereus*. The survival rates of *E. heros* suggested that control efficiency is not solely linked to bacterial concentration, but it also depends on the mechanisms of action and the ability to colonize and interact with the pest.

Keywords: agricultural pest, hemipteran insect, biocontrol, microbial agents, insecticidal activity.

INTRODUCTION

Agriculture plays a fundamental role in food security and economic stability worldwide. It faces significant challenges due to the presence of pests that affect crops, as well as their productivity and quality (ARORA, 2018; SOUTO et al., 2021). Among these pests, *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae), native to the Neotropical Region, is considered one of the main and most abundant in Brazil (SILVA et al., 2012). It is a sucking insect present in different stages of leguminous plant development, causing significant damage and economic losses for farmers, resulting in losses that can exceed R$ 12 billion annually per harvest (SMANIOTTO; PANIZZI, 2015; PORTAL DO AGRONEGÓCIO, 2024).

Effective management of *E. heros* control has seen various strategies developed and implemented over the years, with insecticides being the main method in use nowadays, including neonicotinoids, pyrethroids, and organophosphates (RIBEIRO et al., 2016; TUELHER et al., 2018). The availability of only three active ingredients for its control and the indiscriminate application of these compounds on the plants have caused biodiversity losses, affecting non-target organisms, human health, and providing the presence of resistant pest populations (ADEMOKOYA et al., 2022).

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In this context, the implementation of less impactful control strategies, such as biological control, has gained much importance because it allows the restoration of the biological balance between pests and their natural enemies, resulting in more sustainable insect control and reducing the economic damage (SINDHU et al., 2017).

The biological control agents for *E. heros* include parasitoids, nematodes, fungi, and bacteria. Egg parasitoids are the most studied and implemented method, but their efficiency has decreased considerably due to incorrect use of chemical products, in addition to limitations in the commercial-scale production of parasitoids (CORRÊA-FERREIRA; SOSA-GÓMEZ, 2017; SILVA et al., 2019).

Nematodes and their symbiotic bacteria have been tested against some species of hemipterans, including stink bugs, and have shown promising results, with significant insect mortality rates (MARRERO et al., 2015). However, their efficiency has been compromised by host insect specificity, requiring the use of different strains to control pests in the same crop (FRANCE, 2013).

Although entomopathogenic fungi species like *Metarhizium anisopliae* and *Beauveria bassiana* have been recommended for stink bug control, their effectiveness may be compromised due to the instability of essential environmental conditions for their establishment in the field, such as exposure to ultraviolet radiation, variations in humidity and temperature fluctuations (RESQUÍN-ROMERO et al., 2020; SILVA-SANTANA et al., 2022).

A promising alternative is the use of bacteria from the Bacillaceae family, which have demonstrated proven toxic activity against various groups of insects and mites and can form thermotolerant endospores that are harmless to vertebrates and plants (MONTEIRO et al., 2005). Some species of *Bacillus* genus are also known for their ability to form crystals, which holds promise for pest control, an aspect that has been studied and explored by the market. Furthermore, other species can induce antagonistic responses in plant diseases and promote plant growth (LANA FILHO et al., 2010). Despite the potential of the bacteria in pest control of stink bugs, the quantity of biological products derived from them remains limited, reflecting a lack of research and highlighting the need to invest in this alternative control strategy.

The Reference Laboratory Unit for Biological Control, located at the Advanced Center for Research in Plant Protection and Animal Health of the Instituto Biológico, in Campinas, SP, Brazil, has a collection of Entomopathogenic Bacteria with a wide variety of strains that have not yet been tested against stink bugs. This research allows the development of sustainable pest management strategies that involve the use of bacteria as biological control agents.

MATERIAL AND METHODS

The study was conducted at the Reference Laboratory Unit for Biological Control, located at the Advanced Center for Research in Plant Protection and Animal Health of the Instituto Biológico, in Campinas.

Stink bugs rearing

Brown stink bugs utilized in this study were reared in 20 × 30 cm plastic cages, maintained in a climate-controlled room with conditions set at 25°C and relative humidity of 60 ± 5%, under a 12-hour photophase. To sustain their growth, the stink bugs were fed a natural diet consisting of bean pods (*Phaseolus vulgaris*), peanuts (*Arachis hypogaea*), sunflower seeds (*Helianthus annuus*), and filtered water.

Bacterial isolates

Bacterial strains were obtained from "Oldermar Cardim Abreu" Collection of Entomopathogenic Microorganisms, housed at the Reference Laboratory Unit for Biological Control of Instituto Biológico, stored in cryopreservation tubes at -20 and -80°C with 15 and 20% glycerol, respectively. These isolates were activated by inoculating 1 mL into Schott bottles containing 50 mL of nutrient broth (NB) (beef extract – 1 g/L, yeast extract – 2 g/L, peptone – 5 g/L, and sodium chloride – 5 g/L) and maintained under agitation 150 rpm at 28°C for 72 hours.

Bacterial rates

Initially, a screening of 125 bacteria from the collection was conducted (data not shown), from which the 19 bacteria that stood out as the most efficient, based on stink bug mortality percentages, were selected. Subsequently, these bacteria underwent another test at a concentration of 10%.

For the experiments, each treatment was subjected to six repetitions. Each repetition was represented by a plastic container (250 mL) containing 20 mL of sterile vermiculite, a natural diet composed of bean pods, peanuts, sunflower seeds, and filtered water, along with five adult brown stink bugs and 7 mL of bacteria-diluted to the concentration of 10% in sterile distilled water. The control treatment consisted of only NB medium.

Molecular identification

Molecular identification was performed through polymerase chain reaction (PCR) amplification of the 16S ribosomal gene and specific genes gyrB and rpoD of the bacteria, followed by sequencing and comparison of sequences on the databases EzBioCloud 16S and GenBank.

To begin, bacteria were activated in NB medium, following the previously described methodology, and individually inoculated onto Petri dishes containing solid nutrient agar culture medium (peptone – 5 g/L; HM peptone B – 1.5 g/L; yeast extract – 1.5 g/L; sodium chloride – 5 g/L; agar – 15 g/L), then incubated at 30°C for 24 hours.

Following growth, genomic DNA extraction was carried out following the Cationic hexadecyl trimethyl ammonium bromide (CTAB) protocol (ROMANO; BRASILEIRO, 1999).

The 16S rDNA ribosomal gene was amplified by PCR using the fD1 (5' – AGAGTTTGATCCTGGCTCAG – 3') e rP1 (5' – ACGGTTACCTTGTTACGACTT – 3') primers (WEISBURG et al., 1991). An approximately 880-pb fragment of the gyrB gene was amplified for Bacillus with gyrB-F (5' – GTNYAYCGTGAYGGNAAAATYC – 3') and gyrB-R (5' – GCAGARTCWCCCTCTACRATATA – 3') primers, and an approximately 800-bp fragment of the rpoD gene was amplified for *Serratia* with rpoD-F (5' – TAYATGMGNGARATGGGNACNGT – 3') and rpoD-R (5' – TTNGCYTCNACCATYTCYTTYTT – 3') primers developed by Dr. Ricardo Harakava, scientific researcher at the Laboratory Reference Unit in Applied Molecular Biology of the Instituto Biológico.

Amplification was carried out using a T100 BioRad thermocycler, consisting of a denaturation step at 94°C/2 min, followed by 40 cycles of 94°C/10 s, primer annealing at 60°C/30 s, an extension at 72°C/1 min, and a final extension at 74°C/4 min.

Samples were subjected to electrophoresis on a 1.5% agarose gel. Subsequently, the size and concentration of amplified fragments were verified in a photodocumentator coupled to a ultraviolet transilluminator.

Amplified products were purified by precipitation with PEG 6000 (SCHMITZ; RIESNER, 2006) and sequenced using the Big Dye 3.1 reagent (Applied Biosystems) and the 3,500-xL capillary sequencer (Applied Biosystems). Obtained sequences were compared with strains deposited in EzBioCloud 16S and GenBank. For the construction of phylogenetic trees, the Neighbor Joining method with 1,000 bootstrap repetitions was used in the MEGA 6.0 program (TAMURA et al., 2013).

Survival rates

Survival rates obtained from the test with a 10% bacterial concentration were plotted. The seven most promising strains were selected, and different concentrations of each bacterium were prepared, including 10, 20, 40, 80, and 100% by cell counting using a Neubauer chamber and diluting.

For each concentration, six repetitions were conducted, and the control treatment was performed only with NB medium. Mortality assessments of the stink bugs were carried out over five days, with the counting and removal of dead individuals in each treatment.

The results were documented and utilized to calculate survival rates for each treatment. A pairwise comparison of mortality rate ranks between treatments was conducted using a Wilcoxon rank sum test, incorporating a Bonferroni correction to account for multiple comparisons.

Statistical analysis

The experiments were conducted following a completely randomized design. The data obtained from the bacteria screening were normalized using the formula proposed by ABBOTT (1925) (Eq. 1):

$$\% \text{ mortality} = \frac{\text{number of dead individuals per replication-dead individual in control}}{\text{100-dead individuals in control}} \; x\, 100 \qquad (1)$$

The results were expressed in percentages of mortality (%), transformed using the arcsine square root transformation, and subjected to analysis of variance (ANOVA) to assess the significance of the observed differences between treatments. Additionally, means were compared and analyzed using the SCOTT and KNOTT algorithm (1974) with the statistical software SISVAR.

RESULTS AND DISCUSSION

In the initial screening stage, *E. heros* mortality rates varied between 0 and 53% among the 125 strains evaluated (data not shown). Bacteria that caused more than 30% *E. heros* mortality rates were chosen for subsequent tests at 10% concentration.

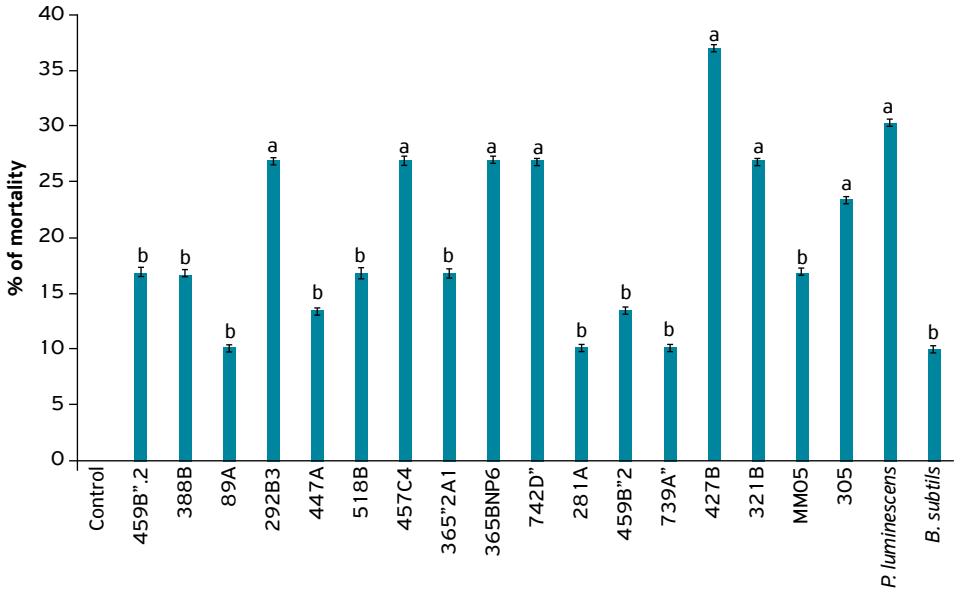

Figure 1. Mortality of *Euschistus heros* in bacterial concentration tests at 10%. Treatments labeled with the same letter do not differ statistically, according to the Scoot & Knott test (0.5%).
Source: Elaborated by the authors.

Among these 19 bacterial strains, 292B3, 457C4, 365BNP6, 742D, 427B, 321B, and *Photorhabdus luminescens* proved to be the most virulent, showing mortality rates of brown stink bugs between 26.67 and 36.67% when bacterial suspensions were tested at 10% of the initial concentration. It is believed that the toxic effect of these bacterial isolates on *E. heros* can be attributed to the invasion of bacterial cells or the production of secondary metabolites with insecticidal activity, as previously noted in the management of other insect pests.

In the molecular analysis of these bacteria, through the sequencing of the 16s ribosomal gene, the highest similarity was found in strains 427B, 292B3, 321B, and 365BNP6 with *Serratia* species, specifically *S. nematodiphila*, *S. marcescens*, and *S. ureilytica*, as shown in Table 1 and Figure 2. Additionally, sequencing the rpoD gene in those isolates allowed for their closest identification with *S. marcescens*, as depicted in Figure 3.

Table 1. EzBioCloud 16S Sequences Matching strains 427B, 292B3, 321B, and 365BNP6, identified through BLAST Analysis of 16S rRNA.

Isolate	Taxon name	Size of the fragment (bp)	Completeness (%)	Similarity (%)	Accession
427B	*Serratia nematodiphila* (DSM 21420)	1,415	100	99.58	JPUX01000001
	Serratia marcescens (ATCC 13880)	1,413	100	99.44	JMPQ01000005
	Serratia ureilytica (NiVa 51)	1,406	99.5	98.94	AJ854062
292B3	*Serratia marcescens* (ATCC 13880)	1,405	100	99.50	JMPQ01000005
	Serratia nematodiphila (DSM 21420)	1,405	100	99.50	JPUX01000001
	Serratia ureilytica (NiVa 51)	1,398	99.5	99.01	AJ854062
321B	*Serratia nematodiphila* (DSM 21420)	1,410	100	99.58	JPUX01000001
	Serratia marcescens (ATCC 13880)	1,408	100	99.44	JMPQ01000005
	Serratia ureilytica (NiVa 51)	1,399	99.5	98.8	AJ854062
365BNP6	*Serratia nematodiphila* (DSM 21420)	1,417	100	99.58	JPUX01000001
	Serratia marcescens (ATCC 13880)	1,415	100	99.44	JMPQ01000005
	Serratia ureilytica (NiVa 51)	1,408	99.5	98.95	AJ854062

Source: Elaborated by the authors.

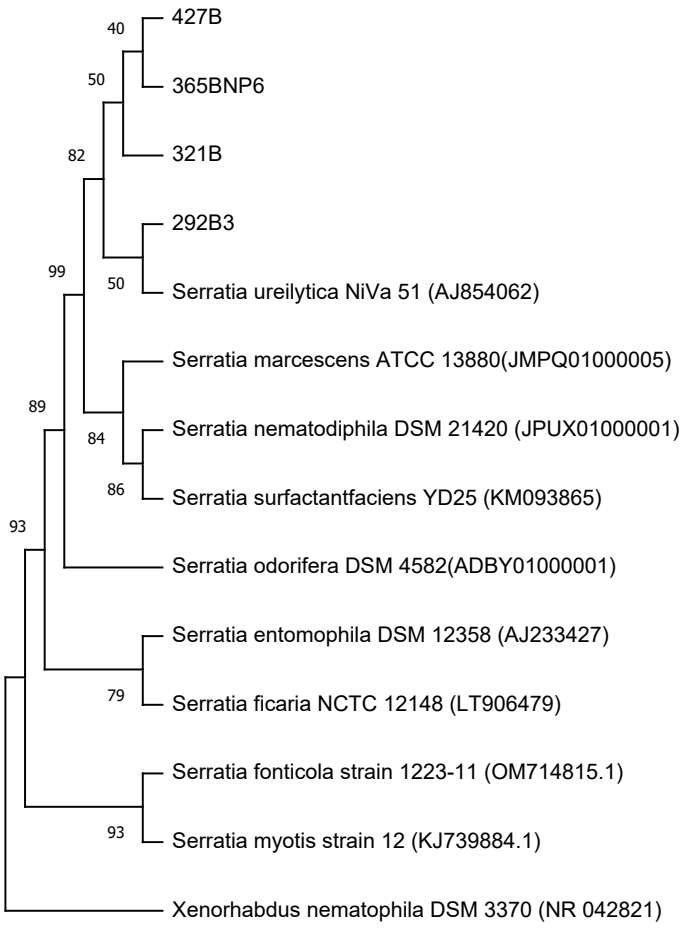

Figure 2. Phylogenetic tree of bacterial isolates 427B, 292B3, 321B, and 365BNP6, constructed from the molecular sequencing of the 16S ribosomal gene.
Source: Elaborated by the authors.

Table 2. GenBank sequences matching strains 427B, 292B3, 321B and 365BNP6, based on gyrB gene analysis.

Isolate	Taxon name	Size of the fragment (bp)	Completeness (%)	Similarity (%)	Accession
427B	*Serratia marcescens* (14BL09)	1,672	100	99.35	AP028476.1
	Serratia marcescens (UMH6)	1,666	100	99.24	CP018926.1
	Serratia ureilytica (CM2016)	1,666	100	99.24	CP091121.1
292B3	*Serratia marcescens* (14BL09)	1,694	100	99.57	AP028476.1
	Serratia marcescens (UMH6)	1,688	100	99.46	CP018926.1
	Serratia ureilytica (CM2016)	1,688	100	99.46	CP091121.1
321B	*Serratia marcescens* (14BL09)	1,781	100	99.49	AP028476.1
	Serratia marcescens (UMH6)	1,775	100	99.39	CP018926.1
	Serratia ureilytica (CM2016)	1,775	100	99.39	CP091121.1
365BNP6	*Serratia marcescens* (14BL09)	1,692	100	99.57	AP028476.1
	Serratia marcescens (UMH6)	1,687	100	99.46	CP018926.1
	Serratia ureilytica (CM2016)	1,687	100	99.46	CP091121.1

Source: Elaborated by the authors.

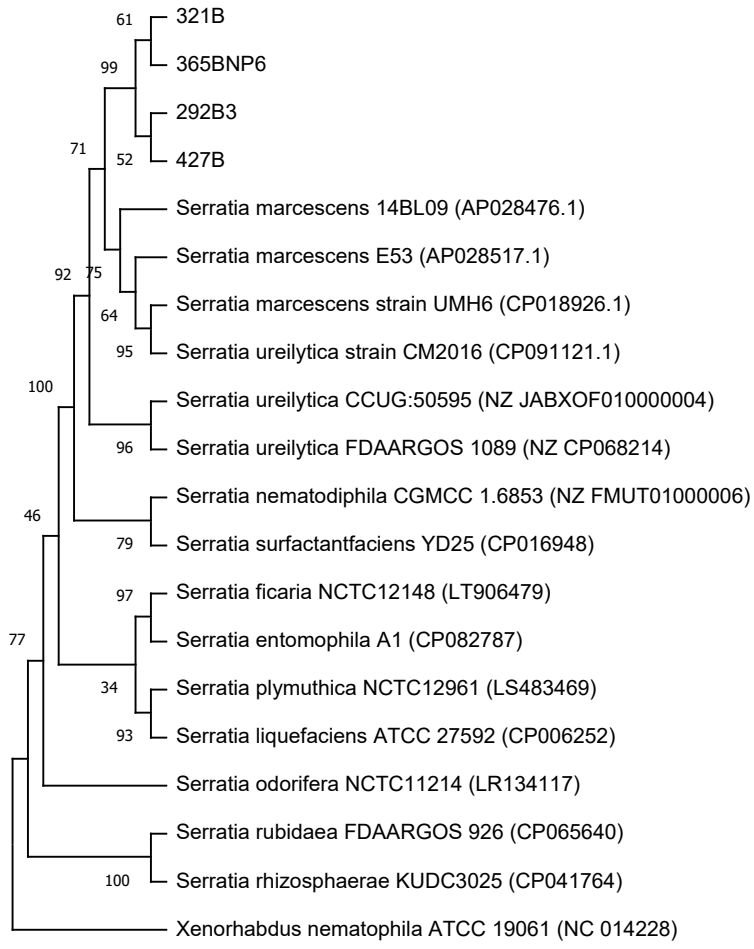

Figure 3. Phylogenetic tree of bacterial isolates 427B, 292B3, 321B, and 365BNP6, based on molecular sequencing of the rpoD gene.
Source: Elaborated by the authors.

Table 3. EzBioCloud 16S Sequences Matching Strains 742D, and 457C4, identified through BLAST Analysis of 16S rRNA.

Isolate	Taxon name	Size of the fragment (bp)	Completeness (%)	Similarity (%)	Accession
742D	*Bacillus toyonensis* (BTC 7112)	1,430	100	100	CP006863
	Bacillus mobilis (0711P9-1)	1,429	100	99.93	MACF01000036
	Bacillus pacificus (EB422)	1,429	100	99.93	KJ812450
	Bacillus paramobilis (BML BC017)	1,428	98.50	99.86	MW674728
457C4	*Bacillus paranthracis* (Mn5)	1,420	100	100	MACE01000012
	Bacillus nitratireducens (4049)	1,420	100	99.93	KJ812430
	Bacillus cereus (ATCC 14579)	1,419	100	99.86	AE016877

Source: Elaborated by the authors.

Furthermore, in the molecular analysis of strain 742D using the 16S ribosomal gene, a greater similarity to *B. toyonensis*, *B. mobilis*, and *B. pacificus* was observed. In contrast, isolate 457C4 showed similarities to Bacillus paranthracis, *B. nitratireducens*, and *B. cereus* sensu stricto, as presented in Table 3. Through the gyrB gene sequencing, it was determined that bacterium 742D exhibited higher similarity to the specimen *B. toyonensis* (BCT-7112), with an identity of 99.51%, and strain 457C4 exhibited similar sequences to *B. cereus* sensu stricto specimens (ATCC 14579), with 99,88% similarity, as evidenced in Figure 5 and Table 4.

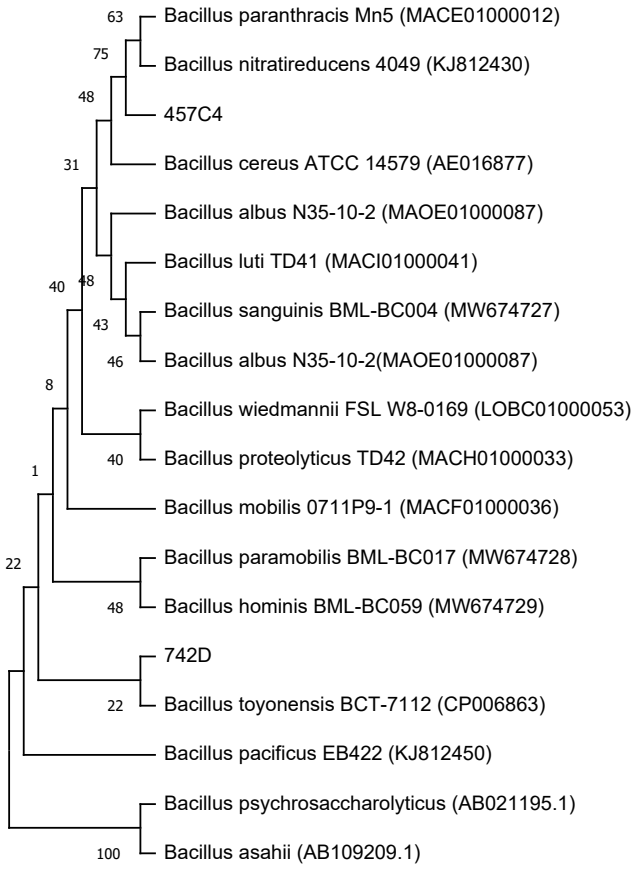

Figure 4. Phylogenetic tree of bacterial isolates 742D and 457C4, constructed from the molecular sequencing of the 16S ribosomal gene.
Source: Elaborated by the authors.

Table 4. GenBank sequences matching strains 742D and 457C4, based on GyrB gene analysis.

Isolate	Taxon name	Size of the fragment (bp)	Completeness (%)	Similarity (%)	Accession
742D	*Bacillus toyonensis* (BTC 7112)	1,480	100	99.51	CP006863
	Bacillus thuringiensis (ATCC 10792)	1,397	100	97.66	CP020754
	Bacillus mycoides (ATCC 6462)	1,125	100	91.64	CP0923291
457C4	*Bacillus cereus* (ATCC 14579)	1,496	100	99.88	CP034551
	Bacillus anthracis (Vollum)	1,153	100	92.26	CP076225
	Bacillus thuringiensis (ATCC 10792)	1,003	100	88.93	CP020754

Source: Elaborated by the authors.

The seven isolates classified in the genera *Photorhabdus, Serratia a*nd *Bacillus* demonstrated the highest potential for controlling the *E. heros* insect pest. In particular, *P. luminescens*, a gram-negative bacterium, is associated with the nematodes (NEPs) *Heterorhabditis bacteriophora* (FISCHER-LE SAUX et al., 1999). Released by NEPs inside the insect, *P. luminescens* recognizes the presence of the amino acid L-proline in the host's hemolymph. This interaction triggers the production of toxins, antibiotics, and other metabolites by the bacterium, leading to the degradation of the insect's hemocoel and preventing the formation of nodules that result in its death (CRAWFORD et al., 2010).

In this study, the strain *P. luminescens* caused a mortality of 30% in *E. heros*. This finding aligns with the research conducted by NANZER et al. (2021), in which two strains of the symbiotic bacterium *P. luminescens* and seven species of *Xenorhabdus* against the stink bugs *E. heros* and *Dichelops melacanthus* were tested. Here, *P. luminescens* was the second-best bacterium, causing a mortality of 37% in the brown stink bug.

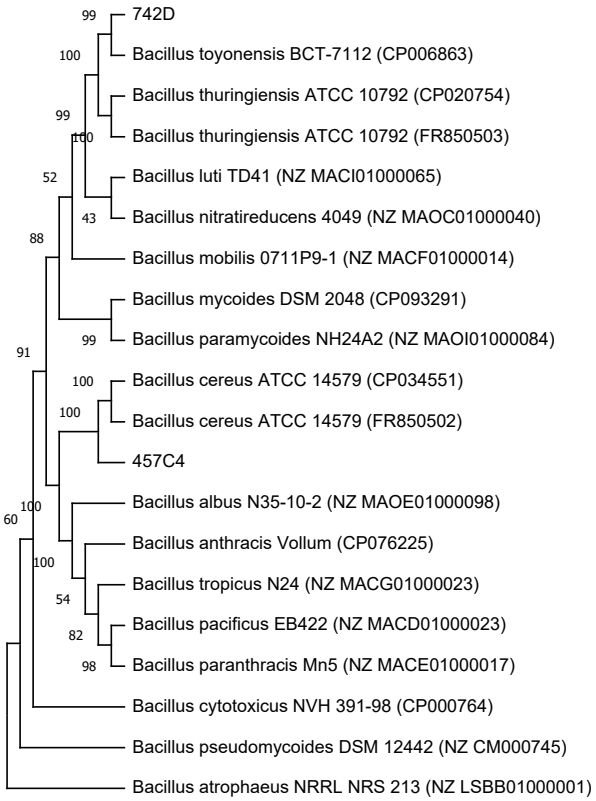

Figure 5. Phylogenetic tree of bacterial isolates 742D and 457C4, based on molecular sequencing of the gyrB gene.
Source: Elaborated by the authors.

However, in another study conducted by MARRERO et al. (2015), *P. luminescens* caused the complete mortality of two species of stink bugs (*Piezodorus guildinii* and *Nezara viridula*). These results contrast with those obtained in *E. heros*, suggesting that the brown stink bug is more resistant to the specific action of *P. luminescens* compared to other stink bug species.

Serratia sp., a gram-negative bacterium with antifungal and antibacterial activity, is naturally present in the soil, and it produces a variety of enzymes and metabolites that impact the survival and reproduction of insects (GRIMONT; GRIMONT, 2006). Specifically, *S. marcescens* exhibit pathogenic and saprophytic characteristics, capable of synthesizing chitinases including ChiA, ChiB, and ChiC, which have been the subject of studies due to their potential use in biological control (SOMEYA et al., 2001).

Extensive research on the biocontrol with *S. marcescens* in different agricultural pests has been conducted. In the context of insects, this bacterium is recognized for inducing bacteremia and rapid mortality in these organisms (GRIMONT; GRIMONT, 2006; ISHII et al., 2014). Recent studies highlighted the promising potential of *S. marcescens* in the control of insects, covering species such as *Meloidogyne incognita*, *Aedes aegypti*, and *Culex quinquefasciatus*, larvae of *Plodia interpunctella*, and *Ephestia Kuehniella* (RAGVENDRAN; NATARAJAN, 2017; BIDARI et al., 2018; HEGAZY et al., 2019).

On the other hand, the *Bacillus cereus* group is composed of eight species, namely *B. anthracis*, *B. cereus stricto sensu*, *B. cytotoxicus*, *B. mycoides, B. pseudomycoides, B. thuringiensis, B. weihenstephanensis*, and *B. toyonensis*, the latter being recently included (JÍMENEZ et al., 2013).

B. toyonensis is a gram-positive, aerobic, endospore-forming bacterium that exhibits antimicrobial compounds (WILLIAMS et al., 2009). Additionally, its ability to promote plant growth, coupled with its safety for humans, animals, and the environment, highlights it as a sustainable alternative to biological control (CONTRERAS-PÉREZ et al., 2019).

Specific studies emphasize the effectiveness of *B. toyonensis* in different biological contexts. BYUNG-RYUN et al. (2018) demonstrated its efficacy in suppressing the bacterium *Pectobacterium carotovorum*. Furthermore, the antifungal activity of *B. toyonensis* has been confirmed against *Botrytis cinerea* (ROJAS-SOLIS et al., 2020). Additionally, other studies revealed significant inhibition of mycelial growth and germination of *Fusarium oxysporum* (SHIN et al., 2023), as well as the evaluation of 93 strains of *Bacillus* sp. for the control of *Alternaria alternata*, highlighting *B. toyonensis* as one of the top four strains with potential for biocontrol (PANE; ZACCARDELLI, 2015).

To date, few studies assessed the insecticidal activity of *B. toyonensis* spores in different insect species, revealing its potential for biocontrol in *Anthonomus grandis* and *Cydia pomonella* (SAUKA et al., 2022). Still, more studies are needed

to explore the potential of this bacterium in other insect pests of the hemipteran group given the limited research available on its use as a biological control agent.

Bacillus cereus, a gram-positive, facultatively anaerobic, endospore-forming bacterium, is commonly found in soil and recognized for its activity in promoting plant growth (SARRÍAS et al., 2002).

Some studies highlight its potential as a biological control agent. The antagonistic effect of *B. cereus* against various phytopathogenic fungi, such as *Penicillium expansum*, *Botrytis cinerea, Penicillium italicum, Geotrichum citri-aurantii,* as well as strong inhibition of *Aspergillus niger* and *Aspergillus carbonarius*, was demonstrated (ABDALLAH et al., 2022; KHADIRI et al., 2023). In addition to this, the antifungal activity of *B. cereus* against *Fusarium oxysporum*, attributing this antifungal effect to the production of hydrolytic enzymes and volatile compounds, was also observed (RAMÍREZ et al., 2022).

Regarding biological control of nematodes, *B. cereus* exerts a strong direct suppression on *Meloidogyne incognita* (YIN et al., 2021). Furthermore, the formation of a biofilm on the surface of the plant roots by this bacterium stimulates growth and provides protection against the pest, which contributes to systemic resistance against pests (NIU et al, 2011; YIN et al., 2021).

This interaction highlights the promising potential of *B. cereus* as a biological control agent. However, current research focuses primarily on the control of phytopathogenic fungi, shedding light to explore its potential in managing insects, especially against hemipteran pests. Future studies in this direction can broaden our understanding of the various roles that *B. cereus* can play in pest management in agriculture, as well as a deeper understanding in host-bacterial interactions.

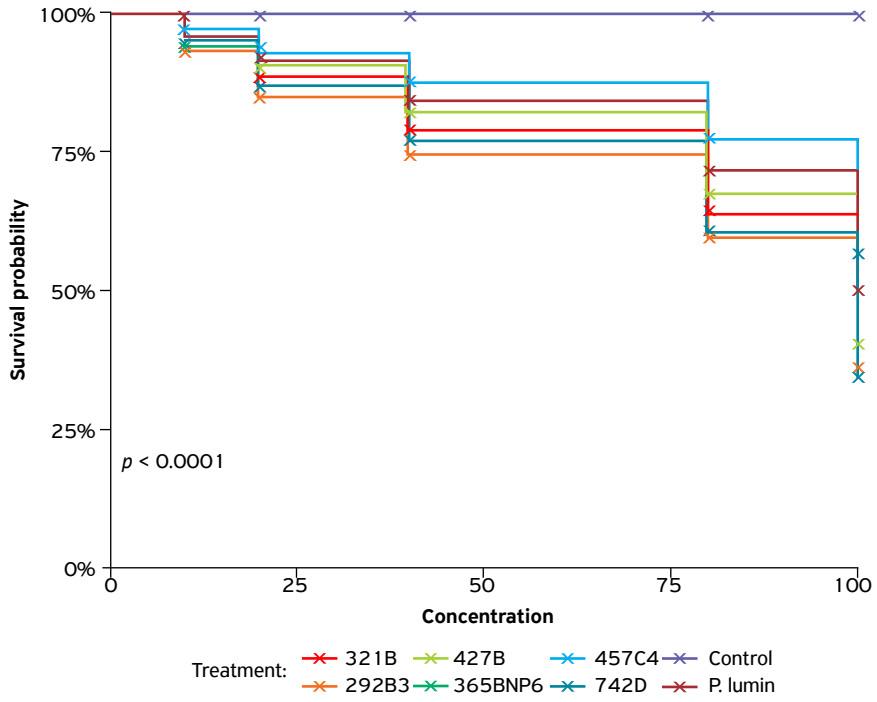

Figure 6. Comparison of survival rates for 10, 20, 40, 80, and 100% concentrations of bacterial isolates 321B, 365BNP6, 292B3, 457C4, 427B, 742D, and *Photorhabdus luminescens*. In analyses, significant differences were not detected between treatments.
Source: Elaborated by the authors.

In general, survival rates did not show significant variation among the different treatments and concentrations, suggesting that the effectiveness in the mortality of *E. heros* adults is not necessarily linked to the concentration of bacteria, but rather to their mechanism of action and their ability to colonize and interact with insect pests and the production of secondary metabolites–toxins–, capable of inhibiting the growth and development of insects, leading to their death. Additionally, it was suggested that enzymes and secondary metabolites produced by bacteria play a crucial role in the control and regulation of pest population (CRAWFORD et al., 2010; STOCK et al., 2017; MAHARANA et al., 2022).

According to the results of this study, it is possible to infer that the bacteria may be valuable as control agents for *E. heros* stink bugs. However, there is a scarcity of available studies on the action of these bacteria as biological control agents of insects, with most of them limited to microbial control. It is imperative to conduct additional research to identify new promising microbiological products in the sustainable management of the brown stink bug and enhance our understanding of the mechanisms of action of these bacteria in controlling this insect (SCHÜNEMANN et al., 2014). This approach will enable the development of more efficient and sustainable control strategies, contributing to the reduction of chemical use and environmental preservation.

CONCLUSIONS

Bacteria are potential agents for biological control in agricultural pest management programs, given their ease of multiplication, application, and effectiveness, aiming to reduce the use of insecticides and preserve the environment. Specifically, the bacteria *S. marcescens* and *B. toyonensis* emerge as promising agents in the biocontrol of *E. heros* pest, demonstrating effectiveness even at low concentrations. These findings highlight the importance of continuing to investigate and evaluate the performance of these bacteria under field conditions, as well as their compatibility with integrated pest management approaches.

AUTHORS' CONTRIBUTIONS

Conceptualization: Esparza-Mora, S.F. **Investigation:** Esparza-Mora, S.F.; Leite, L.G.; Baldo, F.B.; Harakava, R.; Rodríguez-Rodriguez, M.P. **Funding acquisition:** Leite, L.G.; Baldo, F.B. **Methodology:** Esparza-Mora, S.F.; Leite, L.G.; Baldo, F.B.; Harakava, R.; Rodríguez-Rodriguez, M.P. **Writing – original draft:** Esparza-Mora, S.F. **Writing – review & editing:** Esparza-Mora, S.F.; Leite, L.G.; Baldo, F.B.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

Not applicable.

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