



# Characterization of a strain of *Serratia* sp. with ixodicide activity against the cattle tick *Rhipicephalus microplus*

Edgar Castro-Saines<sup>1,2</sup> · Ruben Hernandez-Ortiz<sup>2</sup> ·  
Rodolfo Lagunes-Quintanilla<sup>2</sup> · Guadalupe Peña-Chora<sup>3</sup>

Received: 16 October 2020 / Accepted: 22 June 2021 / Published online: 24 September 2021  
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2021

## Abstract

Cattle ticks are considered the most important ectoparasite in the livestock industry. *Rhipicephalus microplus* causes economic losses both through direct feeding on livestock and through disease transmission. Reports of the failure of chemical ixodicides to control this tick have led to a search for control alternatives, such as bacteria with ixodicide activity. The objective of this work was to select a bacterial strain with ixodicide activity against *R. microplus*. In total, 83 bacterial strains were isolated from soil and dead *R. microplus* specimens, and all strains were evaluated against larvae in a screening test. Bacteria with ixodicide activity were evaluated in larvae and engorged adult female ticks. The larvae were challenged using the larval immersion test (LIT) with 20 µg/mL total protein. The median lethal concentration (LC<sub>50</sub>) for larvae was obtained by using nine total protein concentrations. Engorged adult female ticks were challenged using the adult immersion test (AIT) with six protein concentrations. We evaluated adult mortality on day 10, oviposition rate on day 14 and hatching rate on day 40 after challenge. Only one bacterial strain (EC-35) showed ixodicide activity against larvae and adult *R. microplus*. The highest larval mortality, 52.3%, occurred with a total protein concentration of 40 µg/mL, and the LC<sub>50</sub> was 13.9 µg/mL of protein. In adults, a total protein concentration of 10 µg/mL had the highest mortality (55%), oviposition inhibition (50.9%) and reproductive potential inhibition (52.5%). However, there was no significant effect on hatching. The 16S rRNA gene sequence showed 99% identity of EC-35 with *Serratia* sp.

**Keywords** Bacteria · Ectoparasite · Larval immersion test · Adult immersion test

---

✉ Guadalupe Peña-Chora  
guadalupe.penac@uaem.edu.mx

<sup>1</sup> Facultad de Ciencias Agropecuarias, Universidad Autónoma del Estado de Morelos, Avenida Universidad, 1001, Colonia Chamilpa, CP 62209 Cuernavaca, Morelos, Mexico

<sup>2</sup> Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad, Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Carretera Federal Cuernavaca-Cuautla 8534, Colonia Progreso, CP 62550 Jiutepec, Morelos, Mexico

<sup>3</sup> Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos, Avenida Universidad, 1001, Colonia Chamilpa, CP 62209 Cuernavaca, Morelos, Mexico

## Introduction

*Rhipicephalus microplus* is an ectoparasite affecting cattle and other domestic animals with a worldwide distribution throughout the tropics. This species is important for the livestock industry due to economic losses generated by the transmission of hemoparasites, such as *Babesia bovis*, *Babesia bigemina* and *Anaplasma marginale* (Jongejan and Uilenberg 2004). The main control measure for *R. microplus* is synthetic ixodicides, but their effectiveness is limited due to the appearance of resistant tick populations in the field caused by the frequency of treatments (George et al. 2004). Furthermore, in worldwide tick populations that are simultaneously resistant to organophosphates, pyrethroids, amitraz, macrocyclic lactones (ivermectin), phenylpyrazoles and fluzaron have been reported in such countries as Australia, USA, Mexico, Colombia, Uruguay, Brazil, Argentina and South Africa (Rodríguez-Vivas et al. 2018).

An alternative to control the *R. microplus* population could be the use of microorganisms, such as bacteria. Entomopathogenic bacteria have the ability to secrete toxic proteins and secondary metabolites into the extracellular environment, contributing to the virulence of the bacteria. Genera described to have entomopathogenic effects include *Bacillus*, *Photobacterium*, *Xenorhabdus*, *Pseudomonas*, *Serratia* and *Yersinia* (McQuade and Stock 2018). However, the only species to have been commercialized for pest control are *Bacillus thuringiensis*, *Lysinibacillus sphaericus* and *Serratia entomophila* for use against lepidopteran, coleopteran and dipteran insects, respectively (Lacey et al. 2015). No bacteria-based product is used to control *R. microplus* in the field, and there are only a few reports of trials in vitro (Fernández-Ruvalcaba et al. 2010; Lormendez et al. 2019) with strains of *B. thuringiensis*. Thus, the goals of this work were to isolate bacterial strains from dead *R. microplus* and soil, evaluate them against *R. microplus* larvae and adults, select bacterial strains with ixodicide activity against this tick, and identify the bacteria at the genus level.

## Materials and methods

### Study site

This work was performed in the Plant Parasitology Laboratory at the Biological Center Research of the Universidad Autónoma del Estado de Morelos, Mexico (CIB-UAEM) and in the Tick Laboratory of the National Center of Disciplinary Research in Animal Health and Safety of the National Institute for Forestry, Agricultural and Livestock Research (CENID-SAI/INIFAP) in Jiutepec, Morelos, Mexico.

### Isolation, selection and culture of bacteria

From February to November 2018, in total 160 dead *R. microplus* adult females were collected from 16 cattle-producing ranches located near the coasts of the Gulf of Mexico and Pacific Ocean (from 17° 59' 20.3" N, 90° 56' 14.1" W up to 23° 19' 38.6" N, 97° 58' 24.8" W and from 19° 51' 25.5" N, 103° 46' 24.7" W up to 21° 49' 23.6" N, 105° 11' 2.7" W). The ticks were disinfected with 2% sodium hypochlorite for 10 s and washed twice with sterile distilled water. Subsequently, a tick was placed in a sterile 1.5-mL microcentrifuge tube, where it was fragmented using a sterile dissection needle; then, 1 mL

of Luria–Bertani (LB) culture medium was added, and the tube was incubated for 24 h at 30 °C. Next, to obtain single colonies, a loop of the culture was streaked on solid LB medium in Petri dishes and incubated at 30 °C for 24 h. Subsequently, each single colony was grown individually in a petri dish on solid LB for 72 h. Finally, the culture was mixed with liquid LB and 25% glycerol, and the mixture was placed in a tube in a final volume of 1 mL for cryopreservation at –20 °C until use.

Prior to bioassays with ticks, the tube containing the cryopreserved bacteria was thawed on ice for 15 min and used as the inoculum for 25 mL of LB medium in triplicate. The LB was supplemented with the following additional reagents (in g/L): NH<sub>4</sub>Cl, 1.07; KCl, 1.5; Tris, 18.91; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.19; glucose, 5. The culture was incubated at 30 °C with constant shaking at 200 rpm for 4 days. Then, the culture was concentrated by centrifuging at 4500×g, discarding the supernatant, and resuspending the concentrate in 3 mL of sterile distilled water. Total protein was quantified using the Bradford technique (Bradford 1976).

## Ticks

The bioassays were performed using the Media Joya strain of *R. microplus* originally isolated in Tapalpa, Jalisco, Mexico, which is susceptible to ixodicides and is maintained by artificial infestations on calves within the CENID-SAI/INIFAP facility. In brief, a 120 kg Holstein–Friesian calf was infested with 0.5 g of tick larvae. Twenty-one days later, engorged female ticks were collected directly from the bovine skin and placed in a Petri dish to oviposit.

## Bioassays with tick larvae

The ixodicide activity bioassay was performed for each bacterial strain using three replicates of the larval immersion test (LIT), as described by Klafke et al. (2006). Modifications of the technique consisted of replacing the ixodicide and Triton X-100 with total protein from each bacterial strain and Tween-20, respectively, and that the tick larvae were 30–40 days old. Briefly, in a 1.5-mL microcentrifuge tube, we added 20 µg of total bacterial protein, 1% Tween-20 and sterile distilled water in a final volume of 1 mL. The control group received sterile distilled water and Tween-20 at the same concentration. Subsequently, 200 larvae were immersed for 10 min, and then the larvae were placed in filter paper packages and incubated at 27 ± 2 °C with 80% relative humidity (RH). With the ixodicide activity strain, the mortality rate was evaluated at 1, 2, 4 and 8 days after immersion. Larvae that moved were considered alive, and those that did not show any movement were considered dead.

To determine the median lethal concentration (LC<sub>50</sub>) of the ixodicide activity strain for larvae, nine concentrations of total protein were used (0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20 and 40 µg/mL), and the mortality rate was quantified 4 days after exposure.

## Bioassays with engorged adult ticks

The effect of total protein was evaluated on engorged adult ticks using the adult immersion test (AIT) (Drummond et al. 1973), where the mortality, oviposition and egg hatching (fertility) rates were calculated. Six concentrations of total protein were evaluated (1.25, 2.5, 5, 10, 20 and 40 µg/mL) in sterile distilled water and Tween-20 at a final concentration of 1%.

A total of 45 ticks were used for each protein concentration and were divided into three subgroups of 15 ticks each, and the average weight of each group was similar. The ticks were immersed individually for 10 min in a 1 mL aliquot of the corresponding total protein concentration treatment. The control group was immersed in sterile distilled water with Tween-20. After challenge with total bacterial protein, the ticks were placed individually in 24-well polyethylene plates (Cell Wells; Corning Glass Works, Corning, NY, USA) and incubated at 27 °C and 80% RH for 14 days. Mortality was evaluated 10 days after challenge, and ticks were considered to be dead when they showed darkening of the cuticle and no movement of the Malpighian tubules or legs. The oviposition rate was evaluated 14 days after challenge by weighing the egg mass. The eggs were collected and placed in glass vials with a cotton plug and incubated under the same conditions as adult ticks for 25 days. Next, the hatching rate was evaluated to determine the inhibition of reproductive potential.

### Statistical analysis

The mortality rate of larvae and engorged females, as well as the egg hatching rate, were ln transformed to obtain a normal distribution. The difference in mortality rate between the control and each bacterial treatment in the bioassay with larvae was analyzed using a Student's t-test. The effects of postexposure time and protein concentration on mortality of both larvae and engorged females and on reproductive variables were analyzed by ANOVA followed by a multiple comparison of means using Tukey's test ( $\alpha=0.05$ ), using the SAS v.6.0 (SAS Institute, Cary, NC, USA). The  $LC_{50}$  was calculated by Probit analysis using the Polo Plus 2003 statistical program.

### Identification of bacterial genus

One mL of the sample of bacteria with ixodicide activity after 12 h of growth in LB medium was used for total DNA extraction according to Bravo et al. (1998). Next, 100 ng of DNA was used as a template in each PCR. The identification of bacterial genera was performed by means of PCR amplification and 16S rRNA gene sequencing techniques using the universal oligonucleotide primers, forward 5'-GAGTTTGATCCTGGCTCAG-3' and reverse 5'-GTTACCTTGTACGACTT-3' (Soufiane and Cote 2009). The PCR conditions consisted of an initial denaturation step at 96 °C for 10 min, followed by 35 cycles of denaturation (96 °C, 50 s), annealing (50 °C, 45 s) and extension (72 °C, 60 min) and a final extension step at 72 °C for 10 min. The PCR product was purified using a commercial kit (Wizard® SV Gel and PCR Clean-Up system, Promega, Madison, WI, USA) following the manufacturer's instructions. The purified product was sequenced at the Instituto de Biotecnología of the Universidad Nacional Autónoma de México. The sequence obtained from the 16S rRNA gene was compared with those previously reported in the GenBank database (NCBI) using the Blast program.

### Ethics approval

**Animal welfare statement.** The production of ticks on cattle was approved by the Animal Experimentation and Ethics Committee of the Nacional Center of Disciplinary Research in Animal Health and Safety of the National Institute for Forestry, Agricultural

and Livestock Research (CENID-SAI/INIFAP). This research took ethical and methodological aspects into consideration in accordance with the Mexican regulations on the use, housing and transportation of experimental animals (NOM-062-ZOO-1999 and NOM-051-ZOO-1995).

## Results

### Bioassays with tick larvae

A total of 83 strains were isolated from the dead female tick and soil samples. The mortality was not significantly different from that of the control in 82 of the strains. Only one strain—named EC-35—showed ixodicide activity against larvae of *R. microplus*. The mortality rate of the treated group was higher than that of the control group 1 day after exposing *R. microplus* larvae to 20 µg/mL of total protein of the EC-35 strain, with means of  $26.0 \pm 1\%$  and  $3.1 \pm 0.7\%$ , respectively; on days 2, 4 and 8, there were no significant differences in mortality (Table 1).

The mortality rate of tick larvae exposed to various total protein concentrations differed from that of the control group (Fig. 1). The group exposed to 0.16 µg/mL protein had the lowest mortality rate (17.4%), and the groups exposed to 2.5–40 µg/mL total protein had the highest mortality rates (ca. 50%). There were differences in mortality rate among the four lowest protein concentrations tested (0.16, 0.31, 0.63, and 1.25 µg/mL) increasing from 17.6 to 35.2%, respectively; but no significant differences in mortality rate among the five highest protein concentrations (Fig. 1). Probit analysis estimated an  $LC_{50}$  of 13.89 µg/mL of total protein (Table 2).

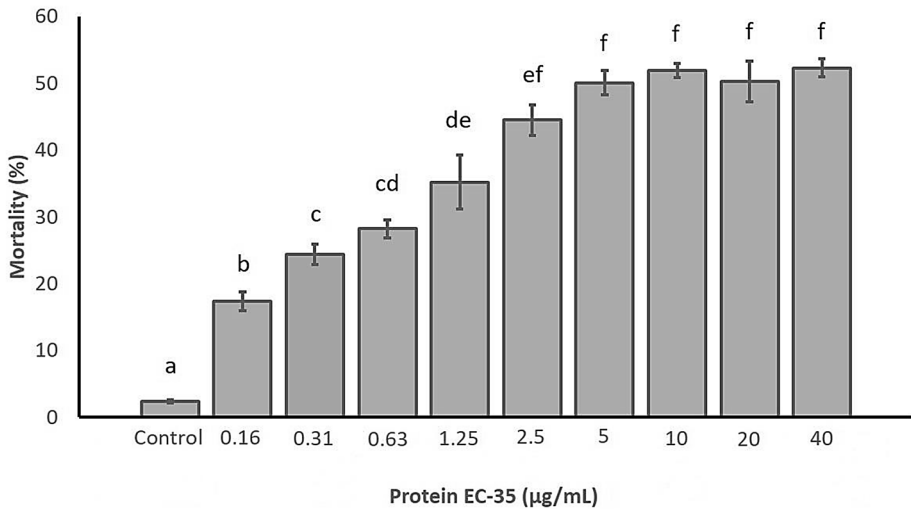
### Bioassays with engorged adult ticks

The mean weight of the engorged female ticks ( $n=15$ ) used for this study was  $4.65 \pm 0.027$  g and did not differ among the subsets assigned to each treatment (Table 3). The effects of the various total protein concentrations of the EC-35 strain on the weight of the egg mass and hatching percentage are shown in Table 3: all treated egg masses were lighter than the untreated control, but egg mass weight did not differ (much) among the treatments, and about half of the eggs hatched in all treatments as well as the control. The treatments affected the mortality rate, reproductive efficiency index, inhibition of oviposition and inhibition of reproductive potential (Table 4).

**Table 1** Mean ( $\pm$ SE) mortality rate of *Rhipicephalus microplus* larvae at various intervals post challenge after immersion in 20 µg/mL total protein concentration of the EC-35 strain

Treatment	Day 1	Day 2	Day 4	Day 8
Control	$0.00 \pm 0.00a$	$2.03 \pm 0.37ab$	$3.02 \pm 0.63b$	$9.63 \pm 1.09c$
EC-35	$26.40 \pm 1.28a$	$34.30 \pm 0.77a$	$39.63 \pm 3.38a$	$35.23 \pm 7.12a$

Means within columns that are followed by a different letter are significantly different (ANOVA:  $p < 0.05$ )



**Fig. 1** Mean ( $\pm$ SE) mortality (%) of *Rhipicephalus microplus* larvae challenged with a range of total protein concentrations of the EC-35 bacterial strain. Means that do not share a letter are significantly different (Tukey's test:  $p < 0.05$ )

**Table 2** Probit analysis values of *Rhipicephalus microplus* larvae challenged with a range of total protein concentrations of the EC-35 strain

n	Slope	LC <sub>50</sub>	95% CI
2758	0.437 $\pm$ 0.034	13.89	8.88 $\pm$ 23.58

LC<sub>50</sub> median lethal concentration, CI confidence interval

**Table 3** Mean ( $\pm$ SE; n=3) reproductive parameters of engorged adult female *Rhipicephalus microplus* challenged with a range of total protein concentrations (TPC) of the EC-35 strain

TPC EC-35 (µg/mL)	Female weight (g)	Egg masses weight (g)	Hatching (%)
Control	4.647 $\pm$ 0.02a	1.992 $\pm$ 0.01a	44.22 $\pm$ 4.16a
1.25	4.659 $\pm$ 0.02a	1.213 $\pm$ 0.04bc	51.82 $\pm$ 3.16a
2.5	4.655 $\pm$ 0.02a	1.322 $\pm$ 0.14bc	52.55 $\pm$ 2.61a
5	4.657 $\pm$ 0.01a	1.337 $\pm$ 0.03bc	53.06 $\pm$ 2.68a
10	4.652 $\pm$ 0.02a	0.979 $\pm$ 0.09c	42.40 $\pm$ 3.00a
20	4.636 $\pm$ 0.01a	1.462 $\pm$ 0.13b	52.85 $\pm$ 2.70a
40	4.667 $\pm$ 0.02a	1.158 $\pm$ 0.09bc	50.76 $\pm$ 2.40a

Coefficients of variation of 0.065, 11.49 and 2.84%, respectively, are shown. Means within a column that do not share a letter are significant different (Tukey's test:  $p < 0.05$ )

### Identification of the bacterial genus in the strain EC-35

The PCR product from the DNA amplification showed an amplicon between 1000 and 1650 bp long, as observed in agarose gel stained with ethidium bromide. The 1432 bp long

**Table 4** Evaluation of the effect of a range of total protein concentrations (TPC) of the EC-35 strain on mean ( $\pm$ SE; n=3) reproductive parameters per group of engorged adult female *Rhipicephalus microplus*

TPC EC-35 ( $\mu\text{g/mL}$ )	Mortality (%)	Oviposition inhibition (%)	IRP (%)
Control	0a	0a	0a
1.25	42.22 $\pm$ 4.45b	39.36 $\pm$ 1.87bc	28.12 $\pm$ 5.10b
2.5	44.44 $\pm$ 9.70b	33.73 $\pm$ 7.50bc	20.28 $\pm$ 10.95b
5	46.66 $\pm$ 10.20b	33.06 $\pm$ 1.78bc	18.64 $\pm$ 6.30b
10	55.55 $\pm$ 8.02b	50.93 $\pm$ 4.49c	52.53 $\pm$ 5.46b
20	33.33 $\pm$ 6.67b	26.44 $\pm$ 6.38b	9.34 $\pm$ 16.64b
40	42.22 $\pm$ 5.89b	42.25 $\pm$ 4.63bc	30.61 $\pm$ 14.87b

*IRP* inhibition of reproductive potential

The coefficients of variation were 8.91, 18.34 and 32.28%, respectively. Means within a column that do not share a letter are significantly different (Tukey's test:  $p < 0.05$ )

sequence of the 16S rRNA gene from the EC-35 strain, when compared with the sequences available in the GenBank (NCBI) database, showed 99% identity with *Serratia marcescens*. MegaBLAST analysis revealed that the EC-35 strain 16S ribosomal RNA gene partial sequence has a 99.86% identity with *Serratia marcescens* strain SYJ1-9 ribosomal RNA gene, partial sequence GenBank accession number KR262852.1. Nucleotide sequence data were submitted to GenBank with accession number MT703954.1. Strains showing no significant differences in mortality were not identified.

## Discussion

In this study, we found that a strain similar to *S. marcescens* showed ixodicide activity against *R. microplus* larvae and adult females. Nevertheless, sequencing of the genomes of *S. marcescens* strains identified 6 or 7 rRNA operons (Li et al. 2015), making this gene a less than ideal candidate for species-level identification. Future studies should be conducted for species-level designation using concatenated amino acid sequences of proteins GyrB and RpoD, which are conserved in bacteria. *Serratia marcescens* has been previously identified in *R. microplus* eggs in a study of the tick bacteriome (Andreotti et al. 2011). Similarly, 16S rRNA sequencing of a group of cultivable bacteria isolated from *Amblyomma cajennense* eggs found 17 species of bacteria, including *S. marcescens* (Machado-Ferreira et al. 2015). Neither of those reports evaluated the lethal effects of this bacterium on ticks.

Different secretions of proteins produced by *Serratia* species, such as pore forming toxins (Hertle 2005), hydrolytic enzymes (Veliz et al. 2017) and biosurfactants (Clements et al. 2019), could be used as biocontrol agents against ticks because they produce hemolysins, chitinases and lipopeptides able to degrade lipids. These proteins could degrade the cuticle of the tick that is composed mainly of chitin and a layer lipid (Hackman 1982). A biocontrol method against *R. microplus* using secretions produced by *Serratia* species could avoid side effects of *Serratia* on the treatment of bovines, including a possible pathogenesis due to the wide host range of *Serratia*, which includes arthropods, humans and (other) mammals.

The *in vitro* ixodicide effect of *Serratia* sp. on *R. microplus* larvae was observed in the initial bioassays using a 20 µg/mL total protein concentration. Larvae that had been subjected to protein from *Serratia* sp. had a 26% mortality rate 1 day after the challenge, and the highest mortality was found on day 4 at 39.6%, although there were no significant differences in mortality among days 1, 2 and 8. Larvae of *R. microplus* cultivated under laboratory conditions have an average life span of 60 days. Larvae used for the bioassays were 30–40 days old. At an intermediate age, their immune system could cope with a probable infection, and only the weakest larval ticks died in the early days after challenge. The isolation of *S. marcescens* from the sea sponge *Haliclona amboinensis* and the sequence of its genome indicate the presence of genes that code for amylases, lipases and proteases (Cheng et al. 2018); these enzymes could be responsible for the degradation of macromolecules present in the tick cuticle, which could allow the bacteria to penetrate the tick. When the tick larvae were challenged with different total protein concentrations of the EC-35 strain, the highest mortality was 52% using a concentration of 40 µg/mL of total protein, whereas the LC<sub>50</sub> was 13.9 µg/mL, a relatively low dose when compared to that reported for other mites and bacteria, such as *Psoroptes cuniculi* challenged with protein from *B. thuringiensis*, where 1.3 mg/mL of total protein was necessary to kill 50% of mites (Dunstand-Guzmán et al. 2015). In assays with *R. microplus* and *P. cuniculi*, the maximum mortality rate only slightly exceeded 50% despite using protein concentrations higher than the LC<sub>50</sub>, whereas in bioassays with *Phyllophaga* larvae fed 10<sup>8</sup> cells of *Serratia entomophila*, the mortality rate was 72% (Nuñez-Valdez et al. 2008). Lepidopteran larvae fed cabbage leaves sprayed with a liquid culture of *S. marcescens* showed 91.7% mortality (Jeong et al. 2010). The differences in the mortality rate caused by these bacteria could be observed because the challenge to ticks in this study was by immersion bath, where the main contact route is through the cuticle, whereas in tests with Coleoptera and Lepidoptera, the bacteria are ingested, which is more likely to enable bacteria to penetrate through the gut.

The virulence of other *S. marcescens* strains has been associated with the hemolytic activity of such proteases as serralysin, chitinases, gelatinases, DNases and siderophore production (Pineda-Castellanos et al. 2015; Raymann et al. 2018; Méndez-Santiago et al. 2021). Hemolysins produced by *S. marcescens* are pore-forming toxins known as Sh1A and Sh1B, which cause lysis of erythrocytes and eukaryotic cells (Hertle 2005). The serralysin metalloprotease allows bacteria to suppress the cellular immunity of *Bombyx mori* by reducing the adhesive properties of hemocytes in this insect (Ishii et al. 2014). Other *Serratia* species also produce metalloproteases, gelatinases, alkaline proteases and chitinases (Matsumoto 2004; Pinna et al. 2009; Kwak et al. 2015). An *S. grimesii* metalloprotease called grimelysin has proteolytic activity on actin (Bozhokina et al. 2011). Our bioassays with engorged adult female ticks using total protein concentrations ranging from 1.25 to 40 µg/mL had a maximum mortality rate of 55.5%, and a protein concentration of 10 µg/mL caused 50.9% inhibition of oviposition and 52.5% inhibition of reproductive potential. Nevertheless, no significant differences were observed among the different total protein concentration treatments with the EC-35 strain. The peak mortality was at a dose of 10 µg/mL (55%), with lower values on either side of this peak (this was also true of oviposition inhibition and IRP). This phenomenon, called hormesis inverted U-shaped, describes the biphasic dose–response relationship exhibiting low-dose stimulation and high-dose inhibition (Calabrese and Baldwin 2002).

*Bacillus thuringiensis* (*Bt*) is considered the most successful biopesticide; it produces protoxins known as Cry and Cyt, which, when ingested by insect larvae, form pores in intestinal cells (Bravo et al. 2011). Engorged female *Argas persicus* ticks were exposed to *B. thuringiensis kurstaki*, resulting in 100% mortality 5 days after being exposed to a



concentration of 1 mg/mL of total protein; *B. thuringiensis israelensis* caused 100% mortality at 2.5 mg/mL, whereas *B. thuringiensis thuringiensis*, at a 5 mg/mL concentration, induced 93.3% mortality; on the other hand, with *Hyalomma dromedarii*, there was 93% mortality using doses as high as 10 mg/mL (Hassanain et al. 1997). Another report indicated that *B. thuringiensis kurstaki* at a concentration of  $10^6$  spores/mL was toxic to engorged *Ixodes scapularis* larvae, with an  $LC_{50}$  of  $10^7$  spores/mL (Zhioua et al. 1999). *Ornithodoros erraticus* ticks artificially fed a mixture of *B. thuringiensis* spores and blood had a 100% mortality rate (Samish and Rehacek 1999). *Bacillus thuringiensis* strains reached up to 80% mortality in engorged female *R. microplus* ticks at a concentration of 1.25 mg/mL 15 days after challenge (Fernández-Ruvalcaba et al. 2010), whereas another trial reported a strain that overexpresses an S-layer protein, which killed 75% of engorged females at a concentration of 300 µg/mL and reached more than 90% inhibition of hatching at 100 µg/mL (Lormendez et al. 2019). The differences in the lethal effect of *B. thuringiensis* strains compared to *Serratia* sp. on *R. microplus* could be because *Serratia* sp. is considered to be an opportunistic pathogen (Mahlen 2011), meaning that it becomes pathogenic when the immune system of the host is weakened. This species even manages to evade the immune response by blocking the expression of genes that encode antimicrobial peptides or enzymes, such as phenol oxidase (Raymann et al. 2018), that act as part of the innate immune system to prevent the invasion of pathogens in invertebrates.

Despite the problem that *R. microplus* represents for the livestock industry, biological control studies using bacteria are very limited. In this work, the effect of *Serratia* sp. on larval ticks and engorged female ticks is described for the first time. Analysis of the metabolites produced by this bacterium is required to identify the possible mechanisms of action on ticks. More studies on biocontrol agents against *R. microplus* ticks are needed, which could be used in integrated control programs.

## Conclusion

*Serratia* sp. isolated from a dead *R. microplus* tick showed an ixodicidal effect of up to 50% against larval ticks and engorged females under in vitro conditions. To the best of our knowledge, this report is the first to describe a lethal effect of *Serratia* sp. on *R. microplus*.

**Acknowledgements** The authors gratefully acknowledge CONACyT (National Council of Science and Technology) for the scholarship granted to Edgar Castro Saines for his doctorate degree (CVU No. 811487). We also acknowledge Casandra Flores Neri and M.Sc. Francisco Martínez Ibañez for their technical assistance. This work is part of a PhD thesis.

**Author contributions** ECS: Methodology, Writing—original draft, Visualization. GPC: Conceptualization, Methodology, Formal analysis, Writing—Review & Editing, Supervision. RHO: Resources, Writing—Review & Editing, Funding acquisition. RLQ: Resources, Funding acquisition.

**Funding** The project was partially financed by the National Institute of Agricultural and Livestock Forestry Research (SIGI No. 10533234452) granted to Dr. Rodolfo Lagunes-Quintanilla.

**Data availability** The material and data sets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## Declarations

**Conflict of interest** The authors declare no competing interests.

## References

- Andreotti R, de Pérez LAA, Down SE, Guerrero FD, Bendele KG (2011) Assessment of bacterial diversity in the cattle tick *Rhipicephalus (Boophilus) microplus* through taq-encoded pyrosequencing. *BMC Microbiol* 11:6
- Bozhokina ES, Tsaplina OA, Efremova TN, Keвер LV, Demidyuk IV, Kostrov SV, Adam T, Komisarschik YY, Khaitlina SY (2011) Bacterial invasion of eukaryotic cells can be mediated by actin-hydrolyzing metalloproteases grimeysin and proteolysis. *Cell Biol Int* 35(2):111–118
- Bradford MM (1976) A rapid and sensitive method for the quantitation of micrograms quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* 72:248–254
- Bravo A, Sarabia S, Lopez L, Ontiveros E, Abarca C, Ortiz A, Ortiz M, Lina L, Villalobos FJ, Peña G, Nuñez-Valdez ME, Soberón M, Quintero R (1998) Characterization of *cry* genes in a Mexican *Bacillus thuringiensis* strain collection. *Appl Environ Microbiol* 64(12):4965–4972
- Bravo A, Likitvivanavong S, Gill SS, Soberón M (2011) *Bacillus thuringiensis*: a story of a successful bioinsecticide. *Insect Biochem Mol Biol* 41:423–431
- Calabrese EJ, Baldwin LA (2002) Defining hormesis. *Hum Exp Toxicol* 21:91–97
- Cheng T, Saidin J, Danish-Daniel M, Gan H, Mat Isa M, Abu Bakar M, Ismail N (2018) Genome sequence of *Serratia marcescens* subsp. *sakuensis* strain K27, a marine bacterium isolated from sponge (*Haliclona amboinensis*). *Genome Announc* 6:e00022-e00118
- Clements T, Ndlovu T, Khan S, Khan W (2019) Biosurfactants produced by *Serratia* species: classification, biosynthesis, production and application. *Appl Microbiol Biotechnol* 103(2):589–602
- Drummond RO, Ernest SE, Trevino JL, Gladney WJ, Graham OH (1973) *Boophilus annulatus* and *B. microplus*: laboratory test of insecticides. *J Econ Entomol* 66(1):130–133
- Dunstand-Guzmán E, Peña-Chora G, Hallal-Calleros C, Pérez-Martínez M, Hernández-Velázquez VM, Morales-Montor J, Flores-Pérez FI (2015) Acaricidal effect and histological damage induced by *Bacillus thuringiensis* protein extracts on the mite *Psoroptes cuniculi*. *Parasit Vectors* 8:285
- Fernández-Ruvalcaba M, Peña-Chora G, Romo-Martínez A, Hernández-Velázquez V, Bravo A, Pérez D (2010) Evaluation of *Bacillus thuringiensis* pathogenicity for a strain of the tick, *Rhipicephalus microplus*, resistant to chemical pesticides. *J Insect Sci* 10:186
- George JE, Pound JM, Davey RB (2004) Chemical control of ticks on cattle and the resistance of these parasites to acaricides. *Parasitology* 129:S353–S366
- Hackman RH (1982) Structure and function in tick cuticle. *Ann Rev Entomol* 27:75–95
- Hassanain MA, El Garhy FM, Abdel-Ghaffar AF, El-Sharaby A, Abdel Megeed NK (1997) Biological control studies of soft and hard ticks in Egypt. I. The effect of *Bacillus thuringiensis* varieties on soft and hard ticks (Ixodidae). *Parasitol Res* 83:209–213
- Hertle R (2005) The family of *Serratia* type pore forming toxins. *Curr Protein Pept Sci* 6:313–325
- Ishii K, Adachi T, Hamamoto H, Sekimizu K (2014) *Serratia marcescens* suppresses host cellular immunity via the production of an adhesion-inhibitory factor against immunosurveillance cells. *J Biol Chem* 289(9):5876–5888
- Jeong H, Mun H, Oh H, Kim S, Yang H, Kim I, Lee H (2010) Evaluation of insecticidal activity of a bacterial strain, *Serratia* sp. EML-SE1 against diamondback moth. *J Microbiol* 48(4):541–545
- Jongejan F, Uilenberg G (2004) The global importance of ticks. *Parasitology* 129:S3–S14
- Klafke GM, Sabatini GA, de Albuquerque TA, Martins JR, Kemp DH, Miller RJ, Schumaker TTS (2006) Larval immersion test with ivermectin in populations of the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari:Ixodidae) from State of Sao Paulo, Brazil. *Vet Parasitol* 142:386–390
- Kwak Y, Khan AR, Shin JH (2015) Genome sequence of *Serratia nematodiphila* DSM 21420T, a symbiotic bacterium from entomopathogenic nematode. *J Biotechnol* 193:1–2
- Lacey LA, Grzywacz D, Shapiro-Ilan DI, Frutos R, Brownbridge M, Goettel MS (2015) Insect pathogens as biological control agents: back to the future. *J Invertebr Pathol* 132:1–41
- Li P, Kwok AHY, Jiang J, Ran T, Xu D, Wang W, Leung FC (2015) Comparative genome analyses of *Serratia marcescens* FS14 reveals its high antagonistic potential. *PLoS ONE* 10(4):e0123061. <https://doi.org/10.1371/journal.pone.0123061>
- Lormendez C, Fernandez-Ruvalcaba M, Adames-Mancebo M, Hernandez-Velazquez V, Zuñiga-Navarrete F, Flores-Ramirez G, Lina-Garcia L, Peña-Chora G (2019) Mass production of a S-layer protein of *Bacillus thuringiensis* and its toxicity to the cattle tick *Rhipicephalus microplus*. *Sci Rep* 9:17586
- Machado-Ferreira E, Vizzoni VF, Piesman J, Gazeta GS, Soares CA (2015) Bacteria associated with *Amblyomma cajennense* tick eggs. *Genet Mol Biol* 38(4):477–483
- Mahlen SD (2011) *Serratia* infections: from military experiments to ocurent practice. *Clin Microbiol Rev* 24(4):755–791

- Matsumoto K (2004) Role of bacterial proteases in pseudomonal and serratial keratitis. *Biol Chem* 385:1007–1016
- Méndez-Santiago EW, Gómez-Rodríguez O, Sánchez-Cruz R, Folch-Mallol L, Hernández-Velázquez VM, Villar-Luna E, Aguilar-Marcelino L, Wong-Villareal A (2021) *Serratia sp.*, an endophyte of *Mimosa pudica* nodules with nematicidal, antifungal activity and growth-promoting characteristics. *Arch Microbiol* 203:549–559
- McQuade R, Stock SP (2018) Secretion systems and secreted proteins in Gram-negative entomopathogenic bacteria: their roles in insect virulence and beyond. *Insects* 9(2):68. <https://doi.org/10.3390/insects9020068>
- Núñez-Valdez ME, Calderón MA, Aranda E, Hernández L, Ramírez-Gama RM, Lina L, Rodríguez-Segura Z, del Gutiérrez MC, Villalobos FJ (2008) Identification of a putative Mexican strain of *Serratia entomophila* pathogenic against root-damaging larvae of Scarabaeidae (Coleoptera). *Appl Environ Microbiol* 74(3):802–810
- Pineda-Castellanos ML, Rodríguez-Segura Z, Villalobos FJ, Hernández L, Lina L, Núñez-Valdez ME (2015) Pathogenicity of isolates of *Serratia marcescens* toward larvae of the scarab *Phyllophaga blanchardi* (Coleoptera). *Pathogens* 4:210–228
- Pinna A, Usai D, Sechi LA, Carta A, Zanetti S (2009) Detection of virulence factors in *Serratia* strains isolated from contact lens-associated corneal ulcers. *Acta Ophthalmol* 89(4):382–387
- Raymann K, Coon K, Shaffer Z, Salisbury S, Moran N (2018) Pathogenicity of *Serratia marcescens* strain in honey bees. *Mbio* 9(5):e01649–e01718. <https://doi.org/10.1128/mBio.01649-18>
- Rodríguez-Vivas RI, Jonsson NN, Bhushan C (2018) Strategies for the control of *Rhipicephalus microplus* ticks in a world of conventional acaricide and macrocyclic lactone resistance. *Parasitol Res* 117:3–29
- Samish M, Rehacek J (1999) Pathogens and predators of ticks and their potential in biological control. *Annu Rev Entomol* 44:159–182
- Soufiane B, Cote JC (2009) Discrimination among *Bacillus thuringiensis* H serotypes, serovars and strains based on 16S RNA gyrB and aroE gene sequence analyze. *Antonie Van Leeuwenhoek* 95:33–45
- Veliz EA, Martínez-Hidalgo O, Hirsch AM (2017) Chitinase-producing bacteria and their role in biocontrol. *AIMS Microbiol* 3(3):689–705
- Zhioua E, Heyer H, Browning M, Ginsberg HS, LeBrun RA (1999) Pathogenicity of *Bacillus thuringiensis* variety *Kurstaki* to *Ixodes scapularis* (Acari: Ixodidae). *J Med Entomol* 36(6):900–902

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.