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PHORESIS BETWEEN *SERRATIA MARCESCENS* AND *STEINERNEMA CARPOCAPSAE* (RHABDITIDA: STEINERNEMATIDAE) DURING INFECTION OF *GALLERIA MELLONELLA* (LEPIDOPTERA: PYRALIDAE) LARVAE.

MARÍA DE JESÚS ORTEGA-ESTRADA[1], M. CRISTINA DEL RINCÓN-CASTRO[2], REGINA BASURTO-RÍOS[1], JORGE TOLEDO[3], AND JORGE E. IBARRA[1]

[1]Departamento de Biotecnología y Bioquímica, CINVESTAV, Irapuato, Gto., Mexico.

[2]Departamento de Alimentos, División de Ciencias de la Vida, Universidad de Guanajuato, Irapuato, Gto., Mexico

[3]Departamento de Entomología Tropical, El Colegio de la Frontera Sur, Tapachula, Chis., Mexico

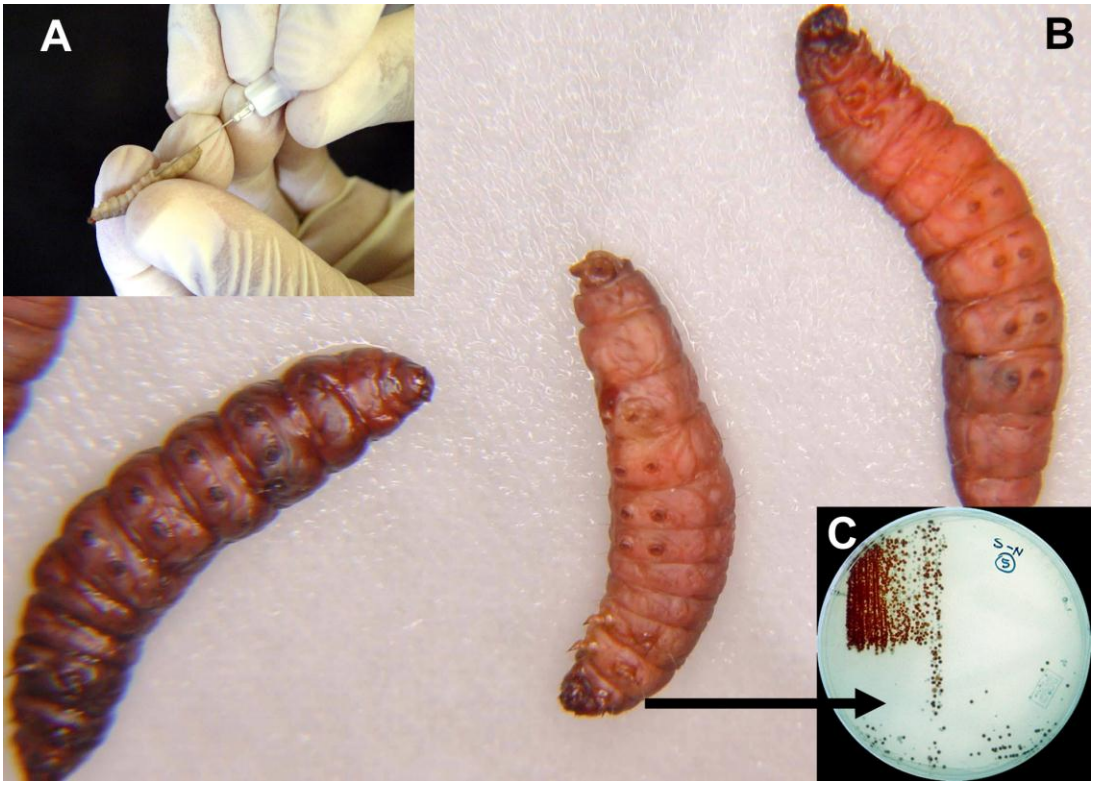
ABSTRACT

Infective juveniles (IJs) of an entomopathogenic nematode strain native to southern Mexico transported an associated bacterium that turned the infected *Galleria mellonella* (L) larvae reddish. The nematode isolate (LBIN-1) was identified as *Steinernema carpocapsae* (Filipjev) by ITS sequence, and its associated bacterium (LBSe-17) as *Serratia marcescens* Bizio by 16S rDNA sequencing. Infectivity of *S. marcescens* LBSe-17 was confirmed by following Koch's postulates on *G. mellonella* larvae. Phoresis of the associated *S. marcescens* bacterium by the nematode into the *G. mellonella* larvae was corroborated by exposing *G. mellonella* larvae to *S. marcescens* either alone or mixed with the nematode. No larval mortality was observed in the first treatment, while 100% mortality was observed in the second treatment. *S. marcescens* was superficially carried by the IJs, as confirmed by surface sterilization of IJs, which caused total larval mortality but no growth of *S. marcescens*. Artificial induction of a similar association was achieved by mixing the *S. marcescens* strain with another *Steinernema* sp. strain (LBIN-2), showing total larval mortality of *G. mellonella* larvae and proliferation of *S. marcescens* in the cadavers. However, 5 consecutive cycles of larval infections showed that colony forming unit

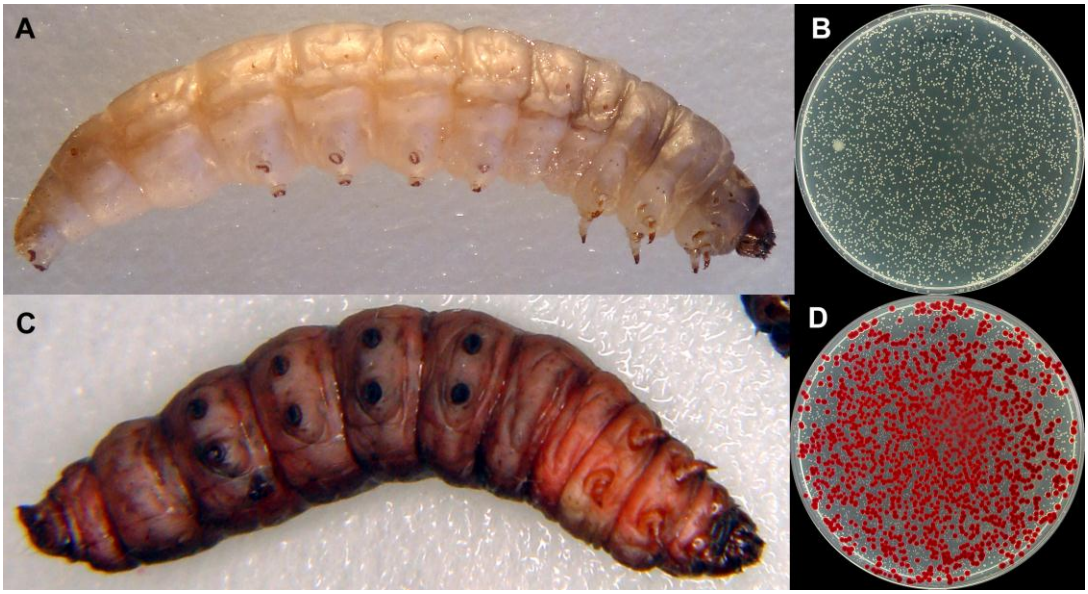
(CFU) counts from the larval cadavers declined sharply in the artificially induced association, while the natural association LBIN-1/LBSe-17 declined, but then tended to stabilize.

RESUMEN

Una cepa de nemátodo entomopatógeno, nativa del sur de México, mostró una bacteria asociada que tornaba rojizas a las larvas de *Galleria mellonella* (L) que infectaba. El nemátodo aislado (LBIN-1) se identificó como *Steinernema carpocapsae* (Filipjev) por secuenciación de su ITS y a la bacteria asociada (LBSe-17) como *Serratia marcescens* Bizio por secuenciación de la subunidad 16S. La infectividad de *S. marcescens* LBISe-17 fue corroborada al aplicar los postulados de Koch sobre larvas de *G. mellonella*. La foresis de la bacteria por el nemátodo al interior de la larva fue corroborada al exponer larvas de *G. mellonella* a la bacteria sola y a una mezcla de la bacteria y el nemátodo. No se observó mortalidad larval en el primer tratamiento mientras que ésta fue del 100%, en el segundo. Se comprobó que la bacteria asociada se transporta en la superficie del nemátodo al infectar larvas de *G. mellonella* con nemátodos esterilizados superficialmente. Todas las larvas fueron infectadas por el nemátodo, pero ninguna desarrolló la infección causada por la bacteria. Una asociación similar se indujo artificialmente al mezclar otra cepa de *Steinernema* (LBIN-2) con *S. marcescens* LBISe-17, provocando el 100% de mortalidad en las larvas infectadas y la proliferación de la bacteria en los cadáveres. Sin embargo, al mantener esta nueva asociación por cinco ciclos consecutivos de infección, los conteos de la bacteria asociada declinaron rápidamente, mientras que éstos, en la asociación natural, tendieron a estabilizarse.

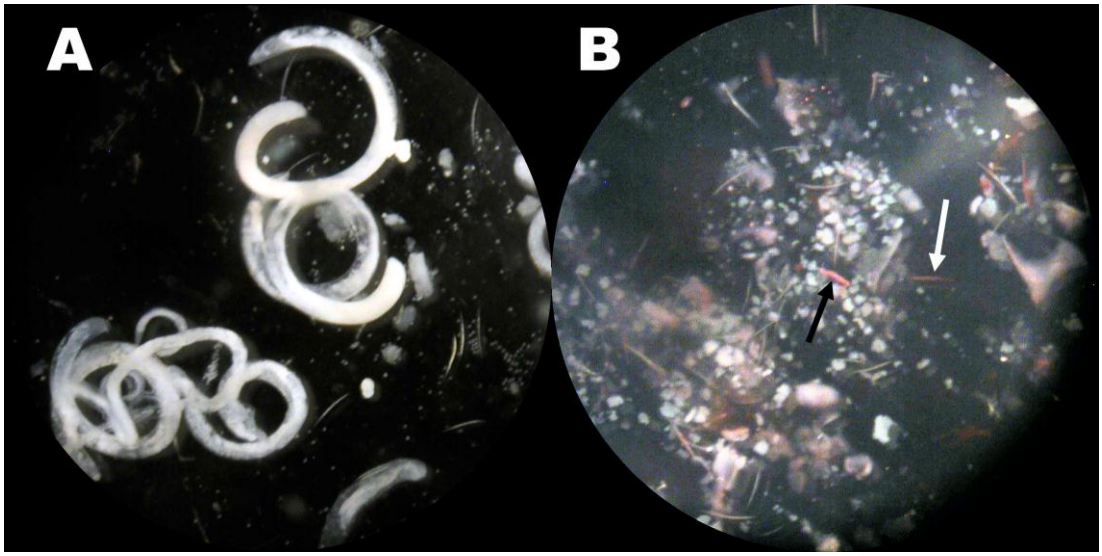


Supplementary Fig. 1. Koch's postulates of the *Serratia marcescens* LBSe-17 strain tested on *Galleria mellonella* larvae. **A.** Infection of a larva with the LBSe-17 strain. **B.** Dead larvae of *G. mellonella* infected with the LBSe-17 strain (24 hours post-infection). C. Re-isolation of the LBSe-17 strain from hemolymph of larval cadavers on *Serratia* medium plates.



Supplementary Fig. 2. *Serratia marcescens* LBSe-17 was carried on the surface of *Steinernema carpocapsae* LBIN-1 infective juveniles (IJs) when the latter infected *G. mellonella* larvae. **A.** Larva infected with surface-sterilized LBIN-1 IJs, i.e., surface sterilization had eliminated *Serratia marcescens* LBSe-17 bacteria from the external surfaces of the IJs. **B.** No LBSe-17 colonies were observed when hemolymph from these infected larvae was inoculated on *Serratia* agar plates. **C.** Larva infected with LBIN-1 IJs that had not been surface-sterilized. **D.** Abundant LBSe-17 colonies were observed when hemolymph from these infected larvae was inoculated on *Serratia* agar plates.

Supplementary Fig. 3. Effect of *Serratia marcescens* LBSe-17 grown on *Galleria melonella* larval cadavers on the production of *Steinernema carpocapsae* LBIN-1 infective juveniles (IJs). **A**. *S. marcescens* growth only noticeable around the spiracles of the larva, which was associated with copious production of IJs (see text). **B.** *S. marcescens* growth very abundant in the *G. melonella* larva, which was associated with a significant decrease of production of IJs (see text).



Supplementary Fig. 4. *Steinernema carpocapsae* LBIN-1 grown *in vitro* on *Xenorhabdus nematophila* and *Serratia marcescens* LBSe-17 cultures. **A.** Adults from an *in vitro* culture of *S. carpocapsae* LBIN-1 being grown on a *X. nematophila* culture. **B.** Dead infective juveniles (IJs) of *S. carpocapsae* LBIN-1 inoculated on a *S. marcescens* LBSe-17 culture. Arrows indicate growth of *S. marcescens* in the IJ cadavers.