

First report of *Bacillus* spp. entomopathogenic to *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae)

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Abstract

Due to the lack of knowledge of pathogenic bacteria to *Melanaphis sacchari*, the objective of this work was to evaluate the pathogenicity of bacterial strains against this aphid. In vitro bioassays were carried out at the Autonomous University of Guerrero in 2019. By intake in a contaminated diet, four strains were evaluated in two treatments of 10 and 100 $\mu\text{g ml}^{-1}$ of total protein. Bioassays showed that the strains were pathogenic to the aphid, resulting in mortality at 48 h of between 35 \pm 8% to 63 \pm 12% at a concentration of 10 $\mu\text{g ml}^{-1}$. While, at a concentration of 100 $\mu\text{g ml}^{-1}$ mortality was around 48 \pm 4% to 90 \pm 7%. The most virulent strains against the aphid at a concentration of 10 $\mu\text{g ml}^{-1}$ were ETH-109 and ETH-117, resulting in mortality of 58 \pm 8% and 63 \pm 12%, respectively. Whereas, to the concentration of 100 $\mu\text{g ml}^{-1}$ the most virulent strain was ETH-109 causing mortality of 90 \pm 7%, followed by the ETH-117 strain with 73 \pm 5% of dead aphids.

Keywords: bacteria, entomopathogenic, plague, sorghum.

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The yellow aphid *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae), is a pest insect native to Africa and is currently distributed in Asia, Australia, Central and South America, about 20 crops belonging to the Poaceae family are its hostess (Singh *et al.*, 2004). In November 2013, this exotic pest was first reported in sorghum crops in Tamaulipas state, Mexico. The affectations were severe, it was estimated that there was 30 to 100% damage to the crop in the spring-autumn seasons, being classified as a new pest of the region (Rodríguez-Bosque y Terán, 2015).

For the control of this insect, field studies showed that products such as Imidacloprid, sulfoxaflor, spirotetramat, thiamethoxam and methamidophos, have an effectiveness greater than 90% mortality (Rodríguez-Bosque y Terán, 2015). On the other hand, biological control is a more environmentally friendly alternative tool for the management of insectile pests, of which there are successful works carried out around the world (Ruiiu, 2015; Lacey *et al.*, 2015).

Generally, the Bacillaceae family has represented the main group of entomopathogenic bacteria, providing the market with the largest number of products and species for biological control. Some of the species representing this family are; *Bacillus thuringiensis*, *Lysinibacillus sphaericus*, *Paenibacillus* spp. and *Brevibacillus laterosporus* (Ruiiu, 2015). Currently, the American company Marrone® Bio Innovations offers two products based on bacteria for the control of aphids, mites and other insects, these being Grandevo® (*Chromobacterium subtsugae*) and Venerate® (*Burkholderia* spp., strain A396) (Toennisson and Burrack, 2018).

However, there are currently few reports of bacteria of the genus *Bacillus* pathogenic to aphids, Torres-Quintero *et al.* (2016) reported that 17 strains of *Bacillus thuringiensis* were pathogenic to peach aphid, *Myzus persicae* (Sulzer); however, to date there are no reports of pathogenic bacteria to the yellow aphid *M. sacchari*. According to the background and due to the lack of knowledge about pathogenic bacteria to this pest, it was set as a target, to evaluate the pathogenicity of different bacterial strains against *M. sacchari*.

To establish the breeding stock of *M. sacchari*, young plants (2 months) of sorghum of the M550 variety were used. Specimens of aphids collected in sorghum crops in the eastern part of Morelos state (18.7669° north latitude, 98.8970° west longitude) were used to infest the plants, placing them in cages made of organza cloth and placed in 1 m³ cages with anti-aphid mesh. The strains to be evaluated were provided by the Laboratory of Plant Parasitology of the Center for Biological Research, of the Autonomous University of the State of Morelos (UAEM), which had been isolated from insect carcasses of Hemiptera order.

For bioassays, bacteria were grown in HCT culture medium (5 g tryptone; 2 g casamino acid medium; 15 g agar) adjusting the pH to 7.5 and sterilizing by autoclave at 120 °C for 20 min to 15-20 psi. After sterilization the culture medium was supplemented with KH₂PO₄, 3.4 g/L; MgSO₄·7H₂O, 0.012 g L⁻¹; MnSO₄·4H₂O, 0.003 g L⁻¹; ZnSO₄·7H₂O, 0.0028 g L⁻¹; Fe (SO₄)₃·7H₂O, 0.02 g L⁻¹; CaCl₂·2H₂O, 0.147 g L⁻¹; and glucose 3 g L⁻¹, which were previously sterilized under the same conditions described above (Torres-Quintero *et al.*, 2016).

The bacteria were sown in Petri box leaving them in incubation at 30 ±1 °C for 72 h. Once sporulated 80% of the bacteria, the spore-crystal complex was recovered in 1 ml of sterile distilled water, adding 1 mM of PMSF (phenyl methane sulfonyl fluoride). The total protein

was quantified using the Bradford (1976) technique at 595 nm. The feeding system used in bioassays was reported by Torres-Quintero *et al.* (2013), 2 ml of diet reported by Toledo-Hernández *et al.* (2018) were added to the container, subsequently placing the Parafilm membrane to turn it over and join it with the other plastic cup, to which the organza cloth was placed to prevent aphids from escaping.

Two treatments, 10 and 100 $\mu\text{g ml}^{-1}$ of total protein added to the diet, were used in pathogenicity bioassays. Subsequently, 15 aphids were placed in each of the feeding systems, assessing mortality at 48 h. The bioassay was left in incubation at 30 ± 1 °C with a relative humidity of $70 \pm 5\%$. Each of the experiments was performed in duplicate with six repetitions each, the negative control was only 30% sucrose diet.

For the visualization of bacteria under the microscope at 100X (Nikon Eclipse 80i), these were planted in Petri boxes with HCT medium and incubated 30 ± 1 °C and at 48 h bacterial biomass preparations were made in slides. The mortality data were analyzed using a one-way Anova, making an average comparison by testing Tukey with a significance level of $p < 0.05$, Sigma Plot 11.0 software was used for statistical analysis. The experimental unit was 15 aphids per feeding system. Two replicates were performed with six replicates for each of the total protein concentrations evaluated.

In a period of two months, it was possible to establish a breeding stock of *M. sacchari* free of parasitized insects, coupled with the fact that this insect has a high reproduction rate. In pathogenicity bioassays at 10 $\mu\text{g ml}^{-1}$ of total protein, it was determined at 48 h that the strains ETH-109 and ETH-117 were the most virulent, as they caused a higher mortality of *M. sacchari*, being around $58 \pm 8\%$ and $63 \pm 12\%$, respectively (Figure 1). Meanwhile, the ETH-116 and ETH-118 strains caused mortality of $35 \pm 8\%$ and $36 \pm 10\%$ of aphids (Figure 1).

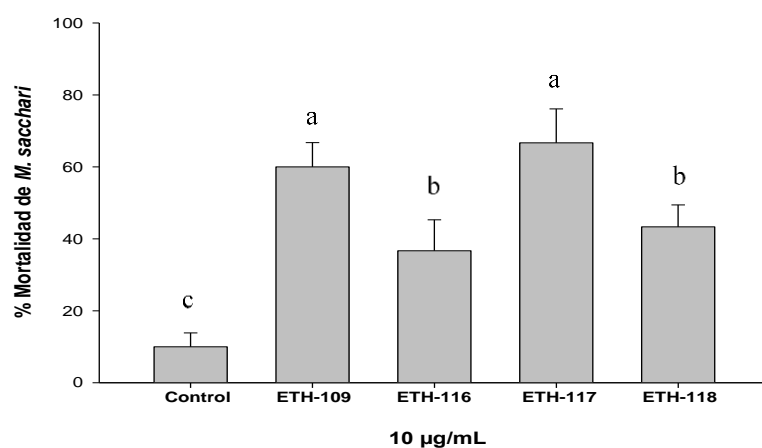


Figure 1. Pathogenicity of strains against *M. sacchari* at a total protein concentration of 10 $\mu\text{g ml}^{-1}$. Bioassay evaluated at 48 h. Bars with different letter denote significant difference according to the Tukey test $p < 0.05$.

On the other hand, in the bioassays of $100 \mu\text{g ml}^{-1}$ of total protein it was determined at 48 h that the ETH-109 strain was the most virulent against *M. sacchari*, causing mortality of $90 \pm 7\%$, followed by the ETH-117 strain with $73 \pm 5\%$ dead aphids (Figure 2). Meanwhile, the ETH-116 and ETH-118 strains resulted in mortality of around $58 \pm 12\%$ and $48 \pm 4\%$, respectively (Figure 2).

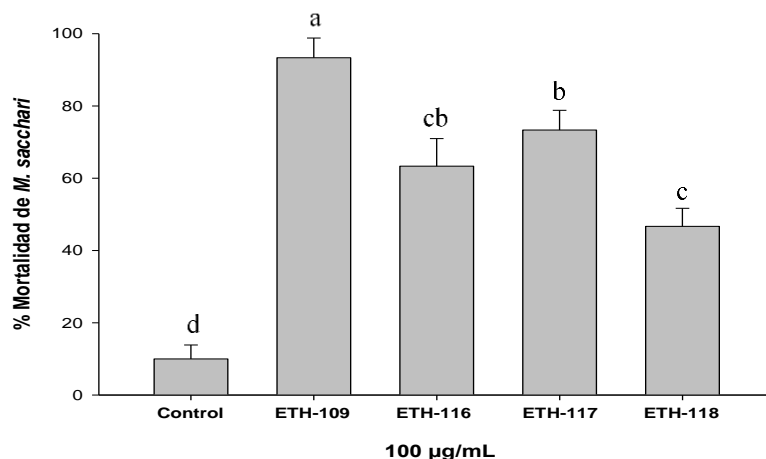


Figure 2. Pathogenicity of strains against *M. sacchari* at a total protein concentration of $100 \mu\text{g ml}^{-1}$. Bioassay evaluated at 48 h. Bars with different letter denote significant difference according to the Tukey test $p < 0.05$.

In the analysis of bacterial samples under the microscope, it was observed that the strains have morphological characteristics of bacillus type, an elongated cane which was of different size in each bacterium. In addition, the cell structure known as sporangium was observed in each of the strains, which consists in the formation of a spore-crystal complex, this being a particular characteristic of the genus *Bacillus* (Figure 3a and 3b).

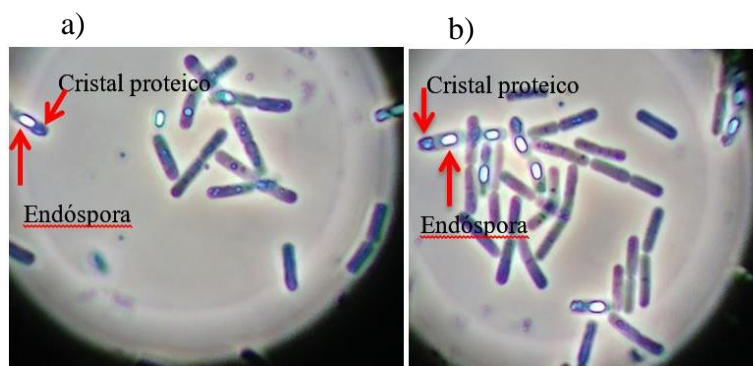


Figure 3. a) ETH-118 strain; and b) ETH-116 strain: present the structure known as sporangium, within which the protein crystal and endospore, particular characteristics of the *Bacillus* genus, are visualized.

To date there is only one study that reports different strains of the pathogenic *Bacillus* genus to the peach aphid, *Myzus persicae* Sulzer. In this work Torres-Quintero *et al.* (2016) assessed the pathogenicity of bacteria isolated from insect carcasses of the Hemiptera Order, strains

corresponding to the species *Bacillus thuringiensis*. Their study consisted of a pathogenicity sieve with approximately 40 strains of *B. thuringiensis* against *M. persicae*, finding that 17 bacteria were pathogenic by total protein intake ($10 \mu\text{g ml}^{-1}$), causing aphid mortality by up to 88% at 72 h. Although the virulence of the strains reported by Torres-Quintero *et al.* (2016) is greater than those found in this study at $10 \mu\text{g m}^{-1}$ (ETH-109 $58 \pm 8\%$ and ETH-117 $63 \pm 12\%$), it is not possible to make a direct comparison, as the strains used in this study were evaluated up to 48 h, in addition, both studies were carried out against different species of aphids.

It should be noted that among aphid species the structure of the digestive system may be different, Ponsen (1991) presented evidence of morphological and histological differences in the digestive system between specimens of the same species. These structural differences influence the susceptibility of the host, increasing or decreasing the virulence of the strains with respect to the host.

Conclusions

It was determined that the four strains evaluated are pathogenic to *Melanaphis sacchari* and belong to the genus *Bacillus*. Meanwhile, the most virulent strains against the aphid at $10 \mu\text{l ml}^{-1}$ of total protein was ETH-109 and ETH-117 ($58 \pm 8\%$ and $63 \pm 12\%$ mortality), respectively. While, at $100 \mu\text{l ml}^{-1}$ the most virulent strain was ETH-109, followed by the ETH-117 strain ($90 \pm 7\%$ and $73 \pm 5\%$), respectively.

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