Journal of Invertebrate Pathology 137 (2016) 54–57

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Short Communication

Larvicidal activity and effects on post embrionary development of laboratory reared *Musca domestica* (Linnaeus, 1758) (Diptera: Muscidae), treated with *Brevibacillus laterosporus* (Laubach) spore suspensions

Vitor dos S.B. Ferreira [a], Isadora da S. Barcellos [a], Isabel N. Carramaschi [a], Jacenir R. Santos-Mallet [b], Margareth M.C. Queiroz [a], Viviane Zahner [a,*]

[a] Laboratório de Entomologia Médica e Forense, Pavilhão Herman Lent, Sala 14, Instituto Oswaldo Cruz/Fiocruz, Av. Brasil, 4365, Rio de Janeiro CEP 21040-360, Brazil
[b] Laboratório Interdisciplinar de Vigilância Entomológica em Díptera e Hemíptera, Instituto Oswaldo Cruz, Fiocruz, Av. Brazil, 4365, Rio de Janeiro CEP 21040-360, Brazil

ARTICLE INFO

Article history:
Received 11 March 2016
Revised 19 April 2016
Accepted 28 April 2016
Available online 6 May 2016

Keywords:
Musca domestica
Brevibacillus laterosporus
Biological control
Public health

ABSTRACT

The application of a spore suspension of *Brevibacillus laterosporus* (Laubach) (strain Bon707), at a concentration of 1.94×10^9 CFU/mL in the diet, induced a level of 70% mortality in larvae of *Musca domestica*. No sublethal effects, upon feeding activity or development were recorded. However, electron microscopic examination of the digestive tract of larvae fed with *B. laterosporus*, revealed cellular vacuolization and cytoplasmic disorganization.

1. Introduction

Musca domestica (Linnaeus, 1758) is a major anthropophilic urban pest, that acts as a mechanical vector of pathogens, can also cause significant annoyance to livestock animals (Malik et al., 2007) and facultative myiasis in wild animals (Dehghani et al., 2012) and humans (Sehgal et al., 2002; Ucan et al., 2011). *M. domestica* can also serve as a phoretic host for the human botfly *Dermatobia hominis* (Linnaeus, 1761) and as such is involved in the process of specific myiasis (Maia and Gomes, 1988).

Chemical insecticides are widely used for the control of *M. domestica* (van Emden and Peakall, 1996). However the excessive use of such compounds may create environmental problems and contribute to the emergence of resistance in insect populations (Shono et al., 2004). In this context, the World Health Organization actively encourages research on new strategies and resources for pest and vector insect control (Mörner et al., 2002).

Brevibacillus laterosporus (Laubach) shows broad entomopathogenic activities (Oliveira et al., 2004) including toxicity against blowflies (Pessanha et al., 2015; Carramaschi et al., 2015) and house flies (Ruiu et al., 2006; Zimmer et al., 2013).

The present study sought to evaluate potential lethal and sub-lethal entomopathogenic effects associated with the use of the Bon707 strain of *B. laterosporus* when added to the diet of *M. domestica* larvae. The possible effects of ingestion of this bacterium upon the integrity of the gastrointestinal tract of larva was assessed through transmission electron microscopy.

2. Materials and methods

2.1. M. domestica colony

The bioassays were performed with fourth generation larvae obtained from adults adapted to laboratory colonies (Laboratório de Entomologia Médica e Forense, Fiocruz) and rearing conditions (27 ± 1 °C, 60 ± 10% R.H. and a 12 h artificial photoperiod).

2.2. Bacterial strain

Individual colonies of *B. laterosporus* strain Bon707, previously demonstrated to be toxic to a variety of invertebrates (Oliveira et al., 2004; Carramaschi et al., 2015; Pessanha et al., 2015) and to hold potential as an entomopathogen of *M. domestica* (Ferreira, 2015), were used to inoculate 45 slants of nutrient agar, prepared in screw-capped test tubes (16 × 125 mm). Cells were incubated until >98% of free spores were obtained (ca. 72 h at 31 °C). Growth from each slant was transferred to 15 mL of distilled autoclaved water by scraping of the agar surface to produce a spore suspension. Suspensions were heated at 80 °C for 15 min to inactivate

* Corresponding author.
E-mail address: vzahner@fiocruz.br (V. Zahner).

http://dx.doi.org/10.1016/j.jip.2016.04.009
0022-2011/

any remaining vegetative cells and to retain the spores. Serial tenfold dilutions of spore suspensions were prepared. A portion of the suspension was used for quantification by light microscopy and inoculation of plates of NA to determine the number of viable spores. The remaining suspension was added to the diets.

2.3. Larvicidal bioassays

Diet for the bioassays consisted of 1 mL of spore suspension mixed with 2.5 g of ground beef and wheat powder mixture (in the proportion of 1 g wheat powder/5 g ground beef). The mixture was placed in a 10 mL plastic recipient which in turn was put into a 50 mL plastic pot containing vermiculite for pupation. This set was covered with organza type fabric. Three different diets were offered to the larvae: pure control (pure diet), water control (1 mL of autoclaved distilled water mixed with diet) and bacterial spore suspension mixed with diet. Newly hatched larvae (L1) (n = 10) were added to the pots containing the diets and placed in a ventilated safety cabinet with incubation at 25 ± 1 °C, 60 ± 10% R.H. and 12 h photoperiod. Each bioassay consisted of five replicates for each control and test group/spore concentration as recommended by Ruiu et al. (2006). The pots were observed daily and larvae that did not abandon the diet or that failed to pupate were recorded as dead. Pupae were collected, weighed and placed individually in test tubes filled to ¼ of their volume with vermiculite and closed with nylon cloth. Then larvae viability was calculated. In the case of emergence of adults, the pupal viability was calculated together with the newly-hatched larvae to adult viability. All experimental steps involved daily observation, until the emergence of adults.

2.4. Transmission electron microscopy (TEM) preparation

To elucidate the possible bioactivity of spore suspension towards L1 larvae, TEM micrographs of the apical portion of epithelial post-midgut cells of larvae were prepared and compared to images produced from larvae fed with control diets. The section used was the post midgut as described by Ruiu et al. (2012), and identified by the proximity to the Malpighian tubules. Fixation of the material was performed following the methods of Sabatini et al. (1963). Contrast, cutting and the micrographs were performed at the Rudolph Barth Electron Microscopy Platform (IOC/Fiocruz) and observed in a JEOL JEM-1011 transmission electron microscope.

2.5. Data analysis

Data from bioassays were interpreted through analysis of variance (ANOVA:$P \leqslant 0.05$) and the Tukey-Kramer test for statistical significance using means and standard deviations with the Graphpad®Instat statistics package. Larval viability was determined for larvae which developed into pupae, pupal viability was calculated for the pupae which became adults and newly-hatched larvae viability was established for larvae which became adults. Mortality was calculated by the formula (Mortality% = 100 − Viability%) and corrected using Abbotťs correction formula (Abbott, 1925) (Mortality treated% − Mortality control%). Lethal concentration 50% (LC_{50}) values were calculated using LogProbit logarithmic regression in the POLOplus statistical package.

3. Results and discussion

Ruiu et al. (2006) observed that *B. laterosporus* was able to reduce the viability of larvae of *M. domestica* by inhibition of feeding, which was reflected in reduced pupal mass. In contrast, in our experiments no significant alteration in the pupae mass was recorded indicating that feeding activity was not affected by the test strain.

Treatment with *B. laterosporus* did not interfere in the development time of *M. domestica* larvae. However, a reduction in the duration of larval period was observed in the pure control, which may be assigned to the relative dryness of the diet substrate, causing premature abandonment of larvae. In addition, treatment did not interfere in pupal development or the time taken for newly-hatched larva to develop into adults (total) stage (Table 1).

Ruiu et al. (2006) found similar results in relation to pupal development although they noticed a decrease in the development time of the larval stage. Riddiford and Truman (1978) suggested that insect development and metamorphosis are hormone mediated which may explain, in part, the absence of significant alterations in the development time of the treated insects in comparison to controls.

The mortality levels observed for treated larvae were superior and statistically significant when compared to the values recorded in either control group. In contrast to the data for sub-lethal effects, the presence of bacteria was seen to have exerted a significant effect upon larval viability. All of the concentrations tested (Table 1), induced mortality when compared to the controls and the mortality levels were found to be concentration-dependent in the case of both the primary larvae and the newly-hatched larvae to adults. In our experiments no statistical difference was obtained in the pupal stage for any of the concentration tested.

Although we did not dissect the larvae to calculate the quantity of spores ingested by individual larvae, our data are in agreement with the findings of Tharwat et al. (1995), Ruiu et al. (2006, 2007) and Zimmer et al. (2013) where the mortality of *M.* domestica, caused by the ingestion of *B. laterosporus*, was dose dependent and was related to the amount of bacteria ingested by the insects. Our data clearly demonstrated that mortality was dose dependent for larvae which ingested the spores and also in the case of newly-hatched larvae to adults. Mortality levels varied from approximately 18% to 72% using the concentrations of 4.8×10^7 spores/g and 7.76×10^8 spores/g, respectively (Table 1). Ruiu et al. (2006) found a LC_{50} value for first instar larvae of 7.20×10^7, while the LC_{50} value recorded for strain Bon707 was 1.66×10^8 spore/g, which can be considered satisfactory for a preliminary study.

Fig. 1 shows the digestive tract of both control and test larvae examined by TEM. Following 48 h of contact with the diet, the epithelial midgut cells of the water control group, presented intact cytoplasm, with little to no digestive vacuoles (Fig. 1A). The microvilli of epithelial cells showed a digitiform shape, an elongated structure with continuous presence along the border. Sections prepared from larva dissected at 72 h after ingestion of the control diet, also showed cytoplasm integrity, limited presence of vacuoles and maintenance of the organization of organelles (Fig. 1B). In contrast, larvae collected 48 h after ingestion of strain Bon707 showed a marked increase in vacuole production and cytoplasmic disorganization (Fig. 1C). The damage observed at 48 h was even more apparent in samples processed at 72 h post ingestion. Numerous vesicles were evident with extensive disorganization of the cytoplasm and organelles, resulting in a spongiform appearance (1D). Ruiu et al. (2012) observed similar cytoplasm vacuolization 12 h after exposure to their strain of *B. laterosporus*, accompanied by mitochondrial damage and the presence of lesions in the cell membrane with extrusion of the cytoplasmic content, together with microvilli damage. The cell damage observed in larvae fed with strain Bon707, is consistent with the type of damage caused by *B. thuringiensis* proteins in different orders of insects (Song et al., 2012).