Journal of Invertebrate Pathology 146 (2017) 69–72

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

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

Laboratory evaluation of *Brevibacillus laterosporus* strains as biocidal agents against *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) larvae

Isabel Nogueira Carramaschi[a], Lorrane de Andrade Pereira[a], Vitor dos Santos Baia[a], Jacenir Reis dos Santos Mallet[b], Margareth Maria de Carvalho 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 21045-900, Brazil*
[b] *Laboratório Interdisciplinar de Vigilância Entomológica em Díptera e Hemíptera, Pavilhão Carlos Chagas, Instituto Oswaldo Cruz/Fiocruz, Av. Brazil, 4365, Rio de Janeiro CEP 21040-360, Brazil*

ARTICLE INFO

Keywords:
Biological control
Entomopathogenic bacteria
Ultrastructure analysis
Chrysomya megacephala
Public health

ABSTRACT

The biocidal activity of three strains of *Brevibacillus laterosporus* upon the post-embryonic developmental stages of *Chrysomya megacephala* was evaluated. Bioassays were performed to verify lethal and sub-lethal effects including ultra-structural changes in the midgut. Among the strains assayed, Shi3 presented the highest larval mortality rates, achieving 70% at a concentration of 1×10^8 spores/g of diet. Transmission electron microscopy revealed intestinal alterations caused by all strains tested. The findings of this study indicate that Shi3 represents a promising tool for use in the biocontrol of *C. megacephala*.

1. Introduction

Brevibacillus laterosporus is a biological control agent (Carramaschi et al., 2015) as it shows broad entomopathogenic activity against various insect orders as well as mollusks and nematodes (Oliveira et al., 2004; Tian et al., 2007). Its broad biotechnological potential is clearly demonstrated via the deposition of a number of patents in recent years (Arnaut et al., 2011; Delrio et al., 2011; Gangavaramu, 2013). Its potential against *C. megacephala*, however, has scarcely been demonstrated as this insect is not a major problem globally but in developing countries. The insecticidal action of different *B. laterosporus* strains seems to involve mechanisms similar to *Bacillus thuringiensis*, such as the interaction between toxins and receptors in the intestinal epithelium of the target insect. Once the bacterial toxins are ingested, these proteins would cause osmotic imbalance and intestinal paralysis leading to inanition, sepsis and death (Schnepf et al., 1998). The deterioration of the larval gut epithelium, disorganization of the cytoplasm and organelles, mitochondrial damage and the presence of lesions in the cell membrane and microvilli damage with extrusion of the cytoplasmic content have been observed on electron micrographs taken on susceptible hosts (Ferreira et al., 2016; Ruiu et al., 2012). Lethal and sublethal effects due to the presence of entomopathogenic bacteria in the insect diet were observed by Ruiu et al. (2006). The broad spectrum of action exhibited by this bacterial species may depend on the production of enzymes and other proteins that can contribute to its biological control properties (Panda et al., 2014; Ruiu, 2013).

The present study aimed to evaluate the lethal and sub-lethal entomopathogenic effects associated with a range of *B. laterosporus* strains previously reported to be active against *C. megacephala*. The ultrastructural damage to the intestinal epithelium of this insect species was assessed by transmission electron microscopy.

2. Material and methods

2.1. Collection of flies and colony maintenance

Collection of flies and colony maintenance were performed according to Carramaschi et al. (2015).

2.2. Bacterial strains and preparation of spore suspensions

Strains IGM 16–92, Bon707 and Shi3 that showed larval mortality rates superior to 50% in preliminary experiments (Carramaschi et al., 2015), were used in this study.

A volume of 500 µL of each 18 h-grown bacterial culture was transferred to test tubes containing 5 mL NYSM medium (Favret and Yousten, 1985) and incubated on an environmental Incubator (ES-20/60, BioSan), shaking at 120 rpm, at 31 °C for 6 h. Thereafter, 1 mL of

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

http://dx.doi.org/10.1016/j.jip.2017.04.009
Received 19 October 2016; Received in revised form 15 April 2017; Accepted 21 April 2017
Available online 22 April 2017
0022-2011/ © 2017 Elsevier Inc. All rights reserved.

each pre-culture was transferred to 1 L flasks containing 225 mL of the same culture medium, at 31 °C, 200 rpm for 72 h, until sporulation exceeded 95%. The sporulated culture was harvested by centrifugation at 25.000*g* for 40 min at 4 °C. The pH of the biomass was adjusted to 5.0 with 0.7% propionic acid (Oliveira et al., 2004). Direct cell observations (light microscopy) and viability counts were performed at 72 h to determine cell numbers and to confirm the growth phase of the culture.

Finally, each preparation of biomass was adjusted with sterile distilled water to obtain the different concentrations used in the bioassays.

2.3. Bioassay

Assays were conducted according to Carramaschi et al. (2015), starting with newly hatched larvae (n = 10) transferred to plastic pots containing 5 g of premixed diet (rotting ground beef) mixed with 4 mL of each bacterial spore suspension. Five replicates were used for each bioassay. Negative control consisted of larval rearing diet mixed with 4 mL water.

2.4. Dissection of insects and ultrastructural analysis by transmission electron microscopy

Changes in the larval midgut at the ultrastructural level, due to feeding on a diet containing approximate 2×10^7 spores/g were evaluated via transmission electron microscopy. Dissections were performed 72 h after feeding on the treated diet, according to Boonsriwong et al. (2011), with 10 third instar larvae used per group.

The dissection of the target midgut region for ultrastructural analyses was performed according to Ruiu et al. (2012). The gut region of interest from each larva was placed in Eppendorf tubes containing fixative solution. Fixation of the material was performed following methods of Sabatini and Barrnett (1963). Contrast, sectioning and the preparation of micrographs were performed at the Rudolph Barth Electron Microscopy Platform (IOC/Fiocruz) using a JEOL JEM-1011 microscope.

2.5. Statistical analysis

Data for mortality levels, adult weight and for larval and pupal development periods were submitted to variance analysis (ANOVA 1; $P \leq 0.05$), followed by mean comparisons by Tukey test at 5% significance level. All statistical analyses were performed with the program InStat (version 3.05, 2000).

3. Results and discussion

Chrysomya megacephala has a wide geographical distribution and may reach high densities in urban centers. It is attracted to open dumps and markets where exposed meat and fish serve as its breeding sites (Dias et al., 2009). This scenario is of particular concern in Brazil, since in some areas sanitation is inadequate (Prado and Guimarães, 1982; Ferreira and Lacerda, 1993).

Previous data obtained by cell growth on Nutrient Agar showed that strains Bon707, IGM16-92 and Shi3 were able to kill larvae of this blowfly (Carramaschi et al., 2015). However, Table 1 presents the results obtained in the bioassays performed in this study using suspensions with different spore concentrations obtained through growth in liquid media, which presents different results from the previous study. It is possible that growth on solid media favored the production of proteins or other compounds that were secreted by bacteria during the first 72 h, that may have been lost when harvesting the sporulated culture grown on a liquid medium. Moreover, culture supernatants of the same toxigenic *B. laterosporus* strains showed no relevant activity toward houseflies or mosquitoes in common with the findings of Ruiu et al. (2007). Treatments with IGM16-92 presented no

Table 1
Larval, pupal, and total mortality (%), average weight (mg), larval, pupal and total development period (days) of third instar *Chrysomya megacephala* larvae after bioassay with different spore concentrations of *Brevibacillus laterosporus* strains IGM 16–92, Bon 707 and Shi 3.

Bacteria (spores/g)	Mortality (%)			Larval weight
	Larval	Pupal	Total	X ± SD (mg)
IGM 16–92				
Ctrl water	8a, b	2.2b	10a	57.60 ± 7.88b
8×10^8	28a	0a, b	28a	55.84 ± 10.91b
4×10^8	22a, b	2a, b	24a	59.10 ± 5.5b
8×10^7	6b	2a, b	8a	54.00 ± 11.9b
4×10^7	14a, b	4.6a, b	18a	57.17 ± 9.96b
8×10^6	10a, b	8.8a	18a	57.15 ± 10.9b
Bon 707				
Ctrl water	18b	17a	32a	63.12 ± 11.96a, b
4×10^8	32ab	8.8a	38a	55.46 ± 8.72c
8×10^7	36ab	3.1a	38a	61.0 ± 7.67a, b, c
4×10^7	46a	3.7a	48a	66.17 ± 5.78a, b
8×10^6	32ab	8.8a	38a	61.67 ± 11.77a, b, c
4×10^6	30ab	11.4a	38a	59.45 ± 6.31a, b, c
Shi 3				
Ctrl water	20a	4a	24a, c	36.74 ± 10.56a
2×10^8	68b	0a	68b, c	37.96 ± 19.72a
1×10^8	70b	8a	78b	26.96 ± 18.66a
2×10^7	28a, b	6a	34a, c	37.15 ± 15.99a
1×10^7	50a, b	10a	60a, c	39.36 ± 8.25a
2×10^6	46a, b	4a	52a, c	37.15 ± 9.58a

X ± SD Means and standard deviation; Ctrl Water = control with distilled water; Means followed by the same letter did not differ amongst themselves and those followed by different letters showed significant difference, when Tukey tests were used. Values for larval weight are mean ± S.D.

significantly difference from control, indicating no biocidal potential of this strain when grown in a liquid medium. Yet, teratological effects were observed (not shown), in seven adults which presented morphological alterations namely undeveloped chest, short legs, reduced and wrinkled wings. Bon707, after growth on NA (previous study), produced higher larval mortality (70.5% at 1.46×10^7 spores/g of diet) compared to 46% larval mortality at 4×10^7 spores/g from the present study. No significant differences in the rates of pupal mortality among the groups tested were observed. The average weight of the third instar (4×10^8 spores/g) gave rise to larvae with statistically significant lower average weight (55.46 mg).

The bioassay with Shi3 at concentrations of 2×10^8 and 1×10^8 spores/g of diet resulted in larval mortality levels of 68% and 70%, respectively, while it killed 51.6% larvae at 1.36×10^8 spores/g of diet obtained from growth on solid medium (Carramaschi et al., 2015). No difference between treated and control groups were observed for pupal mortality. The difference with the results obtained in other studies employing diverse strains confirm the genetic heterogeneity of *B. laterosporus* (Zahner et al., 1999) and also the complexity of its mechanisms of pathogenicity that are considered to be strain specific. Toxicity may be due to spore concentrations but also to proteins or inclusions produced and secreted in different stages of bacteria growth, spore-associated proteins, binary toxins and crystal proteins in the canoe-shaped parasporal body (Ruiu, 2013; Marche et al., 2017), soluble toxins that cause pore formation in target cells (WAR and MIS) (Schnepf et al., 2005) or secreted insecticidal toxins with synergistic action, ISP1A and ISP2A (Boets et al., 2004).

Fig. 1 shows ultrathin sections of the posterior midgut of *C. megacephala* larvae analyzed by TEM. The present study did not assess the progressive effects induced over time, following spore ingestion, however the ultrastructural alterations observed were broadly similar to those obtained with *B. thuringiensis* (Vachon et al., 2012). Fig. 1 also presents a comparison between control and treated larvae. The midgut of the control larvae exhibited a compact monolayer of epithelial cells,

Download English Version:

https://daneshyari.com/en/article/5766992

Download Persian Version:

https://daneshyari.com/article/5766992

Daneshyari.com