Biological Control 50 (2009) 329–335

Contents lists available at ScienceDirect

Biological Control

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

Evaluation of the fungus *Beauveria bassiana* (Deuteromycotina: Hyphomycetes), a potential biological control agent of *Lutzomyia longipalpis* (Diptera, Psychodidae)

Sthenia Santos Albano Amóra [a,*], Claudia Maria Leal Bevilaqua [a,*], Francisco Marlon Carneiro Feijó [b], Mariana Araújo Silva [b], Romeika Hermínia Macedo Assunção Pereira [b], Samara Cardoso Silva [c], Nilza Dutra Alves [b], Fúlvio Aurélio Morais Freire [b], Diana Magalhães Oliveira [c]

[a] *Laboratory of Parasitic Diseases, Programa de Pós-Graduação em Ciências Veterinárias – PPGCV, Universidade Estadual do Ceará – UECE, Brazil*
[b] *Laboratory of Veterinary Microbiology, Universidade Federal Rural do Semi-Árido – UFERSA, Brazil*
[c] *Núcleo de Genômica e Bioinformática Tarsísio Pimenta – NUGEN, Universidade Estadual do Ceará – UECE, Brazil*

ARTICLE INFO

Article history:
Received 11 March 2009
Accepted 21 May 2009
Available online 27 May 2009

Keywords:
Biological control
Vector
Lutzomyia longipalpis
Entomopathogenic fungus
Beauveria bassiana
Visceral leishmaniasis

ABSTRACT

Visceral leishmaniasis is a zoonosis whose primary vector in Brazil is the sandfly *Lutzomyia longipalpis* Lutz & Neiva. Presently, efforts to control the vector have not been effective in reducing the prevalence of disease. A possible alternative to current strategies is the biological control of the vector using entomopathogenic fungi. This study evaluates the effects of the fungus, *Beauveria bassiana* (Bals.) Vuilleman, in different developmental stages of *L. longipalpis*. Five concentrations of the fungus were utilized ranging from 10^4 to 10^8 conidia/ml, with appropriate controls. The unhatched eggs, larvae and dead adults exposed to *B. bassiana* were sown to reisolate the fungus. The fungus was subsequently identified by polymerase chain reaction (PCR) and DNA sequencing. Exposure to *B. bassiana* reduced the number of eggs that hatched by 59% ($P < 0.01$). The longevity of infected adults was 5 days, significantly lower than that of the negative control which was 7 days ($P < 0.001$). The longevity of the adult sandfly exposed to the positive chemical (pyrethroid, cypermetherin) control was less than 1 day. The effects of fungal infection on the hatching of eggs laid by infected females were also significant and dose-dependent ($P < 0.05$). With respect to fungal post-infection growth parameters, only germination and sporulation were significantly higher than the fungi before infection ($P < 0.001$). The identity of the reisolated fungus was confirmed by automated DNA sequencing post-passage in all insect stages. These data show that *B. bassiana* has good pathogenic potential, primarily on *L. longipalpis* larvae and adults. Consequently, the use of this fungus in sandfly control programs has potential in reducing the use of chemical insecticides, resulting in benefits to humans and the environment.

1. Introduction

Visceral leishmaniasis (VL), a systemic disease that is fatal if left untreated, is caused by the obligate intra-macrophage protozoa, *Leishmania chagasi* Cunha & Chagas (syn. *Leishmania infantum*) in Europe, North Africa and Latin America. It is endemic in large areas of the tropics, subtropics and the Mediterranean basin (Chappuis et al., 2007; Lukeš et al., 2007). The dog is the reservoir in domestic and peridomestic settings. Its main vector in Brazil is the sandfly *Lutzomyia longipalpis* Lutz & Neiva (Diptera: Psychodidae) (Rondon et al., 2008).

Sandflies are small, fragile, nocturnally active insects with weak, direct flight capability. Female sandflies require a blood meal to mature the eggs and both sexes also need sugar for energy, obtained principally from vascular tissues of plants. Adult sandfly shelters during the day are dark humid places such as in tree holes and animal burrows or under rocks. The eggs are laid in terrestrial microhabitat rich in organic matter that provides food for the larvae (Alexander, 2000). *L. longipalpis*, specially, are well adapted to living with humans and domestic animals (Rebêlo, 2001) and can resist adverse conditions and exploit new environments, thereby facilitating VL transmission.

Adult sandflies of both sexes can be collected by several methods, either while foraging at night or resting during the day. Immature stages are difficult to find and much remains to be discovered about sandfly breeding sites, a gap in our knowledge that restricts options for vector control (Alexander, 2000). Vector control is based on the residual application of pyrethroid insecticides to areas connected to human cases. However, this strategy has not been effective (Amóra et al., 2006). Also, the use of these chemical insecticides could result in environmental and toxicology problems, and in the selection of strains of insects that are resistant

* Corresponding authors. Fax: +55 85 3101 9840.
E-mail addresses: stheniasantos@yahoo.com.br (S.S.A. Amóra), claudiamlb@yahoo.com.br (C.M.L. Bevilaqua).

1049-9644/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2009.05.004

to chemical agents, as has been observed in Canada (Mwangala and Galloway, 1993) and Argentina (Guglielmone et al., 2001). Therefore, the development of new vector control techniques is necessary to improve the quality of human life (Angel-Sahagún et al., 2005).

The strategy of biological control is a promising alternative for the control of VL. In particular, fungi are highlighted as the main agent in insect control (Feijó et al., 2007). In this context, the fungus, *Beauveria bassiana* (Bals.) Vuillemin (Deuteromycotina: Hyphomycetes), has been isolated from hundreds of insect species (Kaufman et al., 2005), occurring in epizootic form even among Diptera (Alves and Lecuona, 1998). The fungus has a cosmopolitan pattern of occurrence. It can be collected from both insects and soil samples, where it can persist for long periods and can infect the host at all stages of development (Maciel et al., 2005). Studies suggest that this fungus is also pathogenic to leishmaniasis vectors (Warburg, 1991; Reithinger et al., 1997).

In Brazil, the use of entomopathogenic fungi as a means of biological control for Psychodidae dipterans is still in the early stages of evaluation (Maciel et al., 2005) but it could be useful in the integrated management of sandflies, and ultimately benefit public health. To this end, our study evaluated the action of the fungus *B. bassiana* on various developmental stages of *L. longipalpis*.

2. Materials and methods

2.1. L. longipalpis collection and identification

Field-collected *L. longipalpis* were maintained in BOD incubators at 27 °C, 80% RH with a photoperiod of 12 h (Rangel et al., 1985). To promote oviposition, female sandflies were allowed to feed and obtain a blood meal from anesthetized hamsters for 2 h. Forty-eight hours post-feeding, the adult females were individualized in plastic pots measuring 4 cm in diameter and 4.5 cm in height that were coated internally with sterile plaster to maintain moisture. After oviposition, the females were dissected (Aransay et al., 2000) for identification (Galati, 2003). The newly emerged larvae were fed daily with a diet based on rabbit feces and dried and crushed cassava leaves until the pupal stage. After emergence, adults were transferred to nylon tulle cages measuring 20 cm^3 diameters, fed for 3 days with a glucose solution soaked in sterile cotton, and on the 4th day, anesthetized hamsters were provided to obtain blood meals for the females.

2.2. Preparation of B. bassiana inoculum

The *B. bassiana* inoculum was obtained from the strain CL1 (URM-3447), kindly provided by the Mycology Collection of the Department of Mycology, Universidade Federal de Pernambuco, and was originally isolated from *Castnia licus* Drury (Lepidoptera: Castniidae) in Pernambuco State, Brazil. The fungal cultures were kept on PDA medium (Potato Dextrose Agar, Vetec Química Fina, Rio de Janeiro, Brazil) and diluted to prepare concentrations of 10^8, 10^7, 10^6, 10^5 and 10^4 conidia/ml in 0.05% v/v Tween 80. Conidia were quantified by direct counting with an optical microscope using a Neubauer chamber. The average of 5 areas counted per field (n) was multiplied by a fixed factor ($n \times 4 \times 10^6$) to determine the number of conidia in suspension (Alves and Moraes, 1998).

2.3. L. longipalpis susceptibility to B. bassiana

The bioassays, in which eggs, larvae and adults were treated with 5 fungal concentrations, were conducted with 2 control groups: 0.05% v/v sterile Tween 80 (negative control) and 196 μg/ml of the pyrethroid, cypermethrin (positive control) (Feijó et al., 2008). Randomized treatments (21) were performed; 7 for each insect stage, with 3 repetitions in triplicate. Each repetition consisted of 30 samples totaling 630 individuals/repetition.

2.3.1. Egg susceptibility

Thirty eggs were placed in the bottom of each plastic pot similar to those used in the maintenance of the colony. Each fungal suspension (3 ml), cypermethrin or Tween 80 was applied to the inner surface and bottom of each pot using a pipette. The treated pots were then stored in BOD incubators at 27 °C, 80% RH with photoperiod of 12 h. Egg hatching was observed daily and larval mortality was counted 8 days post-treatment.

2.3.2. Larval susceptibility

Thirty first-stage larvae were placed, maintained and infected as described for eggs. They were fed with same diet used for the colony and the larval mortality counts were conducted daily until the pupal stage or until the death of all larvae.

2.3.3. Adult susceptibility

Each repetition consisted of 15 males and 15 females used 48 h post-blood feeding. The insects were first cooled to −2 °C for 5 min for immobilization. Immediately after, they were treated with 3 ml of each fungal suspension, cypermethrin or Tween 80, maintained in nylon cages and fed with glucose solution, as described previously. The adult mortality was counted daily to determine longevity and to count the eggs laid by treated females. Larvae hatched from these eggs were quantified 8 days post-infection to obtain the egg hatching rate.

2.4. B. bassiana growth and microscopic features post-passage in L. longipalpis

The unhatched eggs, larvae and dead adults were sterilized with 3 ml 70% ethanol, 3 ml 4% sodium hypochlorite and 3 ml sterile distilled water for 3 min each. The insects were then seeded individually in PDA medium and the fungal growth was observed during 15 days (Alves, 1998). The analyses of fungal growth parameters were done in triplicate, and microscopic examination followed the methodology outlined in Feijó et al. (2007). These data were compared to the parameters observed before the fungal infection.

2.4.1. Germination

A disc of 5 mm diameter was removed from *B. bassiana* culture with 12 days of growth and transferred to a test tube containing 10 ml 0.05% v/v Tween 80. The suspension was shaken to separate the conidia, diluted and adjusted to 10^4 conidia/ml. From this suspension, 0.1 ml was spread on a petri dish with PDA medium using a Drigalski spatula. The number of conidia in the suspension was determined in a Neubauer chamber at 16 h post-inoculation. A total of 500 conidia per petri dish were counted and categorized into two groups: germinated or those having a germ tube in development, and not germinated.

2.4.2. Vegetative growth

A disc of 5 mm diameter of *B. bassiana* was sown in the center of a petri dish with PDA medium. The growth was measured on day 15 post-inoculation.

2.4.3. Colony counting

The same dilution of *B. bassiana* used for the germination experiments was also used for colony counting. The dilution was spread on petri dishes (0.1 ml), and the colonies were counted on days 3, 6, 9, 12 and 15 post-inoculation.