

Isolation of *Beauveria bassiana* (Bals.) Vuill. (Deuteromycotina: Hyphomycetes) from the Chagas disease vector, *Triatoma infestans* (Hemiptera: Reduviidae) in Argentina

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Abstract

A survey for natural entomopathogenic fungi of the Chagas disease vector *Triatoma infestans* was conducted in five provinces of Argentina since 2001. Nymphs (1.5%) and adults (3.3%) infected with a strain of the fungus *Beauveria bassiana* were found at Dean Funes, Córdoba province, Argentina. Field collected insects that died in the laboratory were maintained in moist chambers and incubated at 22 °C. *Beauveria bassiana* from infected insects was cultured on SDAY media. Pathogenicity tests were conducted with a conidial suspension (1×10^7 conidia/ml) of this isolate on *T. infestans* adults. A mortality rate of 100% was obtained at 15 days post-infection. This is the first record of natural infection of *T. infestans* by *B. bassiana*.

Keywords: Argentina, *Beauveria bassiana*, entomopathogenic fungi, *Triatoma infestans*.

Introduction

Chagas disease is considered by World Health Organization to be one of the greatest scourges in South America. An estimated 16 to 18 million people suffer this disease, while more than 100 million people are at risk of infection [9]. Chagas disease is the most important endemic anthrozoosis in Argentina, with an estimated two million people infected with *Trypanosoma cruzi* Chagas 1909, the causative agent of this disease [14]. *Triatoma infestans* Klug is the main vector of Chagas disease in Argentina [9]. Historically, domestic populations of *T. infestans* have been controlled by synthetic residual insecticides. Due to the detrimental impact of chemical insecticides on the environment and the risk of developing resistance, the role of entomopathogenic microorganisms in vector control need to be examined.

Few entomopathogens have been reported from field collected *T. infestans*. The protozoan flagellate *Blastocrithidia triatomae* Cerisola et al. 1971 [1] and a small RNA virus, Triatoma virus (TrV) [11] are the only natural by occurring entomopathogens reported

from *T. infestans*. Entomopathogenic fungi isolated from insect hosts other than triatomines have been evaluated as potential biological control agents of Chagas disease vectors under laboratory conditions yielded promising results [5, 7, 15].

A survey for entomopathogenic fungi on *T. infestans* was initiated in 2001 in endemic Chagas disease areas of Argentina. The objective of this study was to isolate and identify strains of entomopathogenic fungi infecting *T. infestans* in nature and test the pathogenicity of these fungi.

Materials and methods

Insect collection and culture

Domestic and peridomestic populations of *T. infestans* were sampled in rural areas of five provinces of Argentina: Córdoba, Mendoza, San Juan, San Luis and Santiago del Estero from November 2001 to March 2003. They were transported to the laboratory in sterile plastic containers with folded pieces of paper inside and capped with a fine screen. Triatomines were

maintained at a temperature of 27 ± 1 °C and 60% HR.

Experiments were carried out on an Argentine trypanosome-free colony of *T. infestans* obtained from CEPAVE. Insects were held at 27 ± 1 °C, $60\% \pm 5\%$ HR, a photoperiod of 12:12 h (light:dark) and fed every 10 days on a restrained hen.

Fungal cultures and identification

Field-collected *T. infestans* individuals that died in the laboratory within the 20 days post-collection were held in a moist chamber consisting of a sterile Petri dish with a disc of filter paper moistened with sterile distilled water and incubated at 22 °C. Daily checks were performed up to the fifth day. The filamentous fungi emerging from each insect and identifiable as the genus *Beauveria* were transferred to Petri dishes containing Sabouraud dextrose agar medium with 1% yeast extract (SDAY), and incubated under the same temperature and relative humidity conditions described above. The identification of the species was according to Humber [3]. *Beauveria bassiana* (Bals.) Vuill. isolates from *T. infestans* were identified by macromorphological aspects of the colonies, such as color, diameter, mycelial texture, and for their micromorphological characteristics, as observed under an optical microscope. After identification at the species level the strain was deposited in the Mycological Collection of the Centro de Estudios Parasitológicos y de Vectores as CEPAVE No 044 and at the Fungal Culture Collection of the Instituto de Botánica Spegazzini as LPS No 771. Viability of the conidial fungi after 24 hours was determined following techniques described by Lane et al. [4].

Pathogenicity assays

Three replicates of 10 *T. infestans* adults each were used for bioassays. Insects were not fed during the assay. They were individually submerged in a 5 ml suspension of *B. bassiana* 1×10^7 conidia/ml for 7 seconds in 0.01% Tween 20 (sodium polysorbate). The insects were then air-dried for 1 hour at room temperature. After drying, treated insects were placed individually in small plastic tube (50 × 20 mm), covered by a fine screen and maintained at 27 ± 1 °C and $75\% \pm 5\%$ RH. Controls were handled in a similar manner but insects were submerged in 0.01% Tween 20 without added conidia. Insect mortality was recorded daily. ST₅₀ (median survival time) was calculated using the ViStat Time program [16].

Results and discussion

From a total of 301 adults and 274 nymphs of *T. infestans* collected in 9 localities of five provinces of Argentina, ten (3.3%) adults and four (1.5%) fifth-instar nymphs presented a filamentous fungus identified as *B. bassiana*. All of these insects were collected at Dean Funes (30°24' S–64°21' W), Córdoba province, Argentina, in August 2002. Fungal presence was detected 48 hours after death when exposed to high humidity in moist chambers.

The entomogenous fungus *B. bassiana* has been isolated from different insect hosts in nature in different regions of the world [13], including Argentina [2, 6]. Natural occurrences of entomopathogenic fungi in triatomines are very scarce, and they have been mentioned only from *Linshcosteus* sp. in India [12] and from *Triatoma sordida* in Brazil [8]. A few other fungal species have been reported from the natural flora of the digestive tract of several species of triatomines, although none of them were pathogenic to hosts [10].

Viability of conidia from the *B. bassiana* isolate was 100% at 24 h. The median survival time of *T. infestans* adults exposed to a *B. bassiana* conidial suspension was 6.7 days, and 100% mortality occurred at 15 days post-treatment. Fungal infections were confirmed with light microscopy for all dead insects, and the fungus was re-isolated from cadavers. Previous studies using *B. bassiana* isolated from non-triatomines hosts recorded that this fungus was highly pathogenic to *T. infestans* when exposed at doses of 1×10^8 conidia/ml, recording mortality rates of 93–95% at mean lethal time of 7.2–8.8 days [5].

This is the first record of *B. bassiana* as a natural pathogen of *T. infestans*. More research is needed to determine the occurrence and frequency of *B. bassiana* and other entomopathogenic fungi in all triatomines species known to be vectors of Chagas disease.

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