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# Isolation of *Paecilomyces lilacinus* (Thom) Samson (Ascomycota: Hypocreales) from the Chagas disease vector, *Triatoma infestans* Klug (Hemiptera: Reduviidae) in an endemic area in Argentina

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**Abstract**

A survey for entomopathogenic fungi of the Chagas disease vector *Triatoma infestans* was conducted in two provinces of Argentina from March–December 2003. Field-collected insects that died in the laboratory were individually maintained in moist chamber and incubated at 22 °C. Triatominae adults infected with the fungus *Paecilomyces lilacinus* were found at El Quebracho (27°34′S–64°31′W), Santiago del Estero province, Argentina, in December 2003. *Paecilomyces lilacinus* was cultured and isolated from infected insects in SDAY, PYG and MEA media. Pathogenicity tests were conducted and positive results were recorded. The median survival time (MST) of *T. infestans* exposed to a *P. lilacinus* conidial suspension was 12.8 days, and 100% mortality occurred at 30 days post-treatment. This is the first record of natural infection caused by *P. lilacinus* in *T. infestans* in the world.

**Key words:** entomopathogenic fungi, *Paecilomyces lilacinus*, Triatominae, *Triatoma infestan*s

## Introduction

Chagas disease is considered as one of the greatest scourges in South America by the World Health Organization. It is estimated that 16–18 million people are infected with Chagas disease and about 100 million people are at risk in 21 Latin American countries [1]. In Argentina, Chagas disease is the most important endemic anthropozoonosis, with an estimated two million people infected with *Trypanosoma cruzi* Chagas 1909, the causative agent of this disease [2]. *Triatoma infestans* Klug is the main vector of Chagas disease in Argentina [3].

Historically, domestic populations of *T. infestans* were controlled by synthetic residual insecticides. Due to the detrimental impact of chemical insecticides on the environment and the risk of resistance development, the role of entomopathogenic microorganisms in vector control is especially interesting. Few entomopathogens were reported from field-collected *T. infestan*s, namely the protozoan flagellate *Blastocrithidia triatomae* [4], a RNA virus, Triatoma virus (TrV) [5] and the entomopathogenic fungus *Beauveria bassiana* [6].

A survey for *T. infestans* entomopathogenic fungi was carried out during ten months in endemic Chagas disease areas of Argentina. The goals of this study were the isolation and identification of strains of entomopathogenic fungi infecting *T. infestans* in natural field conditions, and the assessment of the pathogenicity of these fungi on Triatomines.

## Materials and methods

### *Insect collection and culture*

Domestic and peridomestic populations of *T. infestans* were hand collected in rural areas of two endemic Chagas disease provinces of Argentina: Santiago del Estero and Tucumán, from March to December 2003. Adult insects were transported to the laboratory in sterile plastic containers along with folded pieces of paper, capped with fine mesh and maintained at 27 ± 1 °C and 60% RH. Triatomines were identified according to Lent and Wygodzinsky [7].

Experiments were carried out on a trypanosome-free colony of *T. infestans* maintained at CEPAVE. Insects were kept at 27 ± 1 °C, 60% ± 5% RH, 12:12 h photoperiod (light:dark) and fed every 10 days on a restrained hen.

### *Fungal cultures and identification*

*Triatoma infestans* individuals that died in the laboratory within the 20 days post-collection were surface sterilized and held in a sterile culture chamber consisting of a Petri dish (100 mm diameter) with a filter-paper disk that was periodically moistened with distilled water and incubated at 22 °C in the dark. Daily checks were performed during the first five days post-mortem. The filamentous fungi emerging from dead individuals were transferred to Petri dishes containing PYG (peptone yeast glucose), SDYA (Sabouraud Dextrose Agar + 2% Yeast extract) and MEA 2% (Malt extract Agar) + antibiotics [8], and incubated at 26 ± 1 °C and 75 ± 5% RH. The fungal species isolated from *T. infestans* were identified on the basis of macromorphological aspects of the colonies, such as color, diameter, mycelial texture, and through their micromorphological characteristics observed with an Olympus BX41 phase contrast compound microscope. Fungi were identified following Humber [9] and Samson [10].

Fungal isolates were deposited in the Mycological Collection of Centro de Estudios Parasitológicos y de Vectores (CEPAVE) as CEP 071, in the Fungal Culture Collection of the Instituto de Botanica Spegazzini as LPS 827 and in the Collection of Entomopathogenic Fungal Cultures, Ithaca, N.Y., EEUU as ARSEF 7478. Viability of the conidial fungi was determined after 24 h using techniques described by Lane et al. [11].

### *Pathogenicity assays*

Thirty *T. infestans* adults in groups of 10 were used for bioassays. Insects were not fed during the assay. Insects were individually submerged in 5 ml of a suspension of $1 \times 10^7$ conidia/ml in 0.01% (v/v) Tween 20 during 6 s [12]. Submerged insects were air dried for one hour at room temperature under a flow hood. After drying, treated insects were individually placed in small plastic tubes (50 × 20 mm), covered by fine mesh and maintained at 27 ± 1 °C and 75% ± 5% RH. Control individuals were submerged in 0.01% Tween 20 without added conidia and handled in a similar manner. Insect mortality was recorded daily and median survival time (ST50) was calculated using the ViStat Time software program [13].

## Results and discussion

A total of 570 *T. infestans* were collected in 10 localities of Tucumán province and 12 localities of Santiago del Estero province, Argentina. A total of 182 individuals out of 326 insects collected in Tucumán province, and 138 out of 244 insects collected in Santiago del Estero, were placed in a moist chamber and incubated.

Three insects (0.52%) collected at El Quebracho (27°34′S–64°31′W), Santiago del Estero province, Argentina, in December 2003, developed white external mycelia that changed to red vinaceous through sporulation. Sporulation was observed 72 h after death, afterwards the insects were exposed to high humidity in moist chambers (Figure 1). The fungus was identified as *Paecilomyces lilacinus* (Thom) Samson, based on macro and micromorphological characteristics (Figure 2) as described by Samson [10].

The viability of *P. lilacinus* conidia was 93.83% at 24 h. The median survival time of *T. infestans* adults exposed to a *P. lilacinus* conidial suspension was 12.8 days, and 100% mortality occurred at 30 days post-treatment. No mortality occurred among the control insects.

Presence of *P. lilacinus* was confirmed under phase contrast light microscope in all dead insects, and the fungus was re-isolated from insect cadavers.

Natural occurrence of entomopathogenic fungi in triatomines is very scarce. Thus far, two entomopathogenic fungi were reported in triatomines, namely *Beauveria bassiana* in *Linshcosteus* sp.

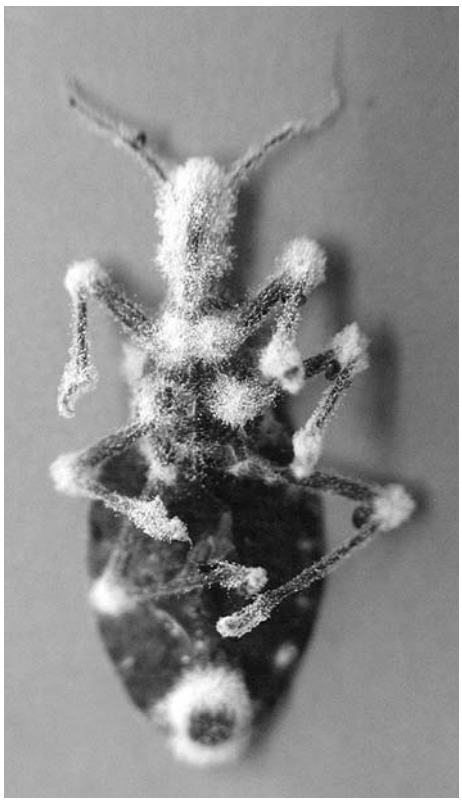

*Figure 1. Triatoma infestans* infected with *Paecilomyces lilacinus* at 10 days post-mortem.

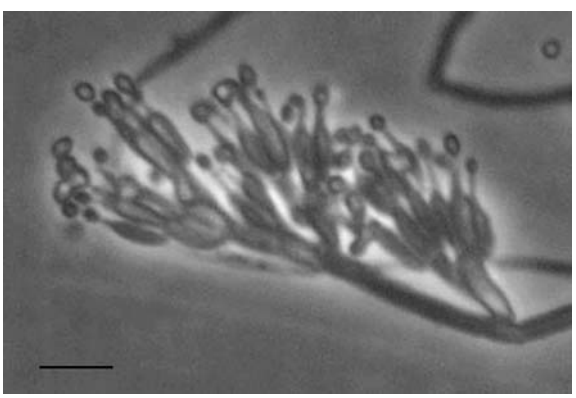

*Figure 2.* Conidiogenous cells (phialides) and conidia (Bar = 8 *μm)*.

from India [14], *Rhodnius pallescens* from Colombia [15] and *T. infestans* from Argentina [6], and *Evlachovaea* sp. in *Triatoma sordida* from Brazil [16]. A few other fungal species were reported from the natural flora of the digestive tract of several species of triatomines, although none of them were pathogenic to their hosts [17, 18].

The entomopathogenic fungus *P. lilacinus* was isolated from diverse insect hosts worldwide, including cimicids [2], weevils, silk worms, bugs, spittle-bugs and nematods [19]. There is only one previous record of *P. lilacinus* in Argentina; it was found infecting cicadellids in Chaco province [20]. This is the first record of *P. lilacinus* as a natural pathogen of *T. infestan*s.

Other entomopathogenic fungi isolated from non-triatomine insect hosts, *Metarhizium anisopliae* and *B. bassiana*, were assessed as potential biological control agents for Chagas disease vectors under laboratory conditions, with promising results [21–23]. Isolates of *B. bassiana* from non-triatomine insect hosts were reported to be highly pathogenic to *T. infestans* when these were exposed at doses of $1 \times 10^8$ conidia/ml, producing mortality rates of 93–95% with a median survival time of 7.2–8.8 days [22]. Marti et al [6] evaluated a native *B. bassiana* isolated from field-collected *T. infestans* hosts in Argentina. This isolate was highly pathogenic to *T. infestans*, causing 100% mortality rates and a median survival time of 6.7 days when the insects were exposed at $1 \times 10^7$ conidia/ml doses [6]. Although the MST of *T. infestans* exposed to *P. lilacinus* ($1 \times 10^7$ conidia/ml) is almost twice the

MST (12.8 days) of insects exposed to *B. bassiana*, similar 100% mortality rates were produced by both fungal species. Given the relatively long life cycle of *T. infestans* (approximately one year), both *P. lilacinus* and *B. bassiana* represent interesting potential biocontrol agents, in spite of the relatively long MST of the former. Further research focused on the effects of this pathogen on both adults and immature stages will contribute to the design of biocontrol strategies.

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