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Fungal flora of the digestive tract of *Triatoma infestans* (Hemiptera: Reduviidae) from Argentina

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Summary: A survey of the fungal microbiota of the digestive tract of *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) adults was carried out. Insects captured in the field from different provinces in Argentina, as well as individuals reared in artificial colonies, were used for dissection. Axenic cultures of the fungal species were identified and were deposited with mycological collections at La Plata, Argentina. A total of 33 fungal species, with the exception of three that were mycelia sterilia, belonging to 11 genera were identified. Thirty two species belonged to Ascomycota (Eurotiomycetes and Sordariomycetes) and one to Zygomycota (Zygomycetes). The genera with the greatest number of species were *Penicillium* (15), *Aspergillus* (5), and *Cladosporium* (2). Among the isolated fungi, some of the species were entomopathogenic or pathogens of humans and other animals.

Key words: Argentina, digestive tract, fungal microflora, insect, symbionts, *Triatoma infestans*.

Resumen: Flora fúngica de tractos digestivos en *Triatoma infestans* (Hemiptera: Reduviidae) en Argentina. En el presente estudio se realizó un relevamiento de la flora fúngica microbiana en tractos digestivos de adultos de *Triatoma infestans* (Klug) (Hemiptera: Reduviidae). Se disecaron insectos capturados del campo en diferentes provincias Argentinas, así como también se utilizaron individuos de una colonia artificial. Fueron realizados cultivos axénicos de las especies fúngicas aisladas, los que fueron identificados y luego depositados en las colecciones de hongos entomopatógenos del CEPAVE La Plata, Argentina. Fueron identificadas 33 especies fúngicas perteneciente a 11 géneros. Treinta y dos especies pertenecen a Ascomycota (Eurotiomycetes y Sordariomycetes) y una a Zygomycota (Zygomycetes). Los géneros con mayor número de especies fueron *Penicillium* (15), *Aspergillus* (5), y *Cladosporium* (2). Entre los aislamientos fúngicos, algunas de las especies encontradas son entomopatogénicas o patógenas de humanos y otros animales.

Palabras clave: Argentina, tractos digestivos, insecto, microflora fúngica, simbiontes, *Triatoma infestans*.

Introduction

Triatomines are obligatory hematophagous insects and vectors of *Trypanosoma cruzi*, (Chagas, 1909), the etiological agent of the Chagas-Mazza disease that is very important and widespread in Argentina and Brazil, as well as in other Latin-American countries, which affects approximately 16 to 18 million people.

The microbial flora of the digestive tract of Triatomines has been reported previously (Moraes *et al*., 1993; Moraes *et al*., 1998; Moraes *et al*., 2000; Moraes *et al*., 2001b) and also from others bloodsucking insects such as mosquitoes (Moraes *et al*., 2001a) and other groups of social insects (Gilliam & Prest, 1972, 1977). Nonetheless the fungal flora of Triatomines has been poorly studied, and most references are from Brazil (Moraes *et al*., 1998; Moraes *et al*., 2000; Moraes *et al*., 2001b). Little is known about mechanisms of interaction between the microbial flora on *T. cruzi* in *T. infestans,* and even less about their potential for possible use in biocontrol of these insects.

The overall objectives of this study were to search for the fungal flora, to isolate and to identify fungal species living in the digestive tract of *T. infestans* adults, as well as to try to determine if any variability exists in the prevalence and presence of these fungal

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species in insects from different regions of Argentina.

Materials and Methods

Insect rearing in the laboratory:

Triatoma infestans insects were reared at CEPAVE (Centro de Estudios Parasitológicos y de Vectores) laboratory facilities. These were held at 27 ± 1 C and 60% RH and a photoperiod of 12:12 h (light:dark). They were fed every 10 days on a restrained chicken.

Insect collections:

Peridomestic populations of *T. infestans* were sampled in rural areas of four provinces of Argentina: Mendoza (San Gabriel LS 32º 19´ 47.7´´ LW 67º 55´ 26.8´´), San Luis (El Zampal LS 32º 21´ 17.9´´ LW 66º 40´ 8.2´´), Salta (Corralito LS 22º 55´ 12´´ LW 63º 31´ 0.3´´) and Santa Fé (El Nochero LS 28º 14´ 0´´ LW 61º 32´ 2.1´´), from November 2004 to March 2005. Individual insects were transported to the laboratory in sterile plastic containers with folded pieces of paper to allow movement and capped with a fine screen. They were maintained under similar conditions in the laboratory. Prospecting of the fungal flora was done from the digestive tracts of ten individuals of *Triatoma infestans* (five females and five males) reared at the triatomines laboratory facilities at CEPAVE. The same procedure was used with ten individuals of *T. infestans* captured in the field from the provinces of Santa Fe, Mendoza, San Luis and Salta, Argentina.

Insect dissection:

The insects were sacrificed after anesthetizing them with a piece of cotton imbibed with chloroform inside cylindrical glass tubes, in a laminar flow hood chamber. They were submerged in successive washes in the following order: 70% ethanol for 30 sec, 1% sodium hypochlorite for 30 sec and two successive washings of 1 min in sterile distilled water, to eliminate any external microbial flora. Each group of insects was dissected using the methods of Lacombe and Range (1997), by retrieving the digestive tract from the esophagus down to the rectum with sterile forceps. The same procedure was used for the triatomines collected from the four provinces of Argentina and for the laboratory-reared insects at CEPAVE, La Plata.

Fungal cultures and identification:

The digestive tracts were individually homogenized for 2 min in a glass tissue grinder containing 1 mL of sterile saline solution (0.9% NaCl). An aliquot of 0.2 mL of each sample was inoculated onto PYG medium (peptone glucose agar + yeast extract) + antibiotics (choramphenicol and gentamicin) in 100 mm diam Petri dishes. They were incubated at ± 24 C in darkness.

Three replicates of each treatment were used, consisting of 10 samples of digestives tracts of *T. infestans* and three controls (0.2 mL sterile saline solution only) for each one of the five experiments. Quantification of colony-forming-units (CFU) of the fungi was conducted up to a total of 15 days after culture incubation. The isolated fungal colonies were inoculated onto different media: PYG (peptone yeast glucose agar), SDYA (Sabouraud dextrose agar + 2% Yeast extract) MEA 2% (Malt extract agar), and PDA (potato dextrose agar) for identification using appropriate literature (Barron, 1968; Carmichel *et al*., 1980; Ramirez, 1982; Barnett & Hunter, 1987; Domsh & Gams, 1993). Fungal material was mounted in Aman's lactophenol-cotton blue 0.01 % and observed under a Zeiss MC63 microscope.

Fungal isolates were preserved in Malt extract agar (AM) and Sabouraud dextrosa agar (SDYA) + 2% Yeast extract and deposited in the Entomopathogenic Culture Collection of CEPAVE, La Plata, Argentina.

Results

Eleven fungal genera including 30 species out of 274 fungal strains were identified and isolated, from five different localities, in decreasing order: *Penicillium* (15), *Aspergillus* (5), *Cladosporium* (2) *Alternaria* (1), *Arthridium* (1), *Acremonium* (1), *Beauveria* (1), *Mucor* (1), *Phoma* (1), *Staphylotrichum* (1), *Talaromyces* (1) and mycelia sterilia. The control culture samples showed no contamination and no fungal growth.

The most frequently found species were *Cladosporium cladosporioides* (Fresenius) de Uries (four locations), *Cladosporium sphaerospermum* Penzig (three locations) *Talaromyces* affinis *helicus* (Raper et Fenell) Benjamin (two locations) and *Aspergillus terreus* Thom (two locations). Mycelia Sterilia (dematiaceous and hyaline) were present in the isolated specimens, and after being spread onto

culture media many of them remained sterile and were allocated as mycelia sterilia (D or H).

From the ten analyzed triatomine samples from San Luis, the fungi identified were *Aspergillus flavus* Link and two species of *Penicillium* (Table 1).

Of the ten analyzed individuals from the insect colony at CEPAVE six of them (three females and three males) presented CFUs belonging to six genera -*Alternaria alternata* (Fries) Keissler, one species of *Aspergillus,* two species of *Cladosporium, Mucor hiemalis* Wehmer, one species of *Penicillium* and *Phoma glomerata* (Corda) Wollemweber et Hochapfel- (Table 1).

Individuals of *Triatoma infestans* collected from Mendoza presented CFUs belonging to five genera - *Arthridium sphaerospermum* (Corda) M. B Ellis,

Table 1. Total quantification of colony-forming-units of microbial fungal flora isolated from the digestive tracts of adult insects from populations of *Triatoma infestans* in Argentina.

	Laboratory	Field			
		Salta	San Luis	Mendoza	Santa Fe
Alternaria alternata	4 (4)*				
Arthrinium phaeospermum				2 (2)*	
Acremonium aff *kiliense*					51 (8)*
Aspergillus aff *fumigatus*	1 (1)*	1 (1)*			1 (1)*
A. aff *versicolor*		4 (1)*		12 (3)*	
A. fumigatus					2 (2)*
A. flavus			1 (1)*		
A. terreus		3 (1)*			1 (1)*
Beauveria bassiana		1 (1)*			
Cladosporium cladosporioides	1 (1)*	7 (4)*		19 (1)*	8 (4)*
C. sphaerospermum	11 (5)*			45 (7)*	1 (1)*
Sterile dematiaceous mycelium				1 (1)*	1 (1)*
Sterile hyaline mycelium					1 (1)*
Mucor hiemalis	1 (1)*				
Penicillium aff *brasileanum*				2 (1)*	
P. aff *frecuentans*				35 (1)*	1 (1)*
P. aff *janthinellum*		1 (1)*			
P. aff *purpurogenum*					10 (3)*
P. aff *rubrum*					1 (1)*
P. aff *thomii*			2 (2)*		
P. griseofulvum					1 (1)*
P. sp 1			2 (1)*		
P. sp 2				5 (2)*	
P. sp 3				1 (1)*	
P. sp 4				8 (2)*	
P. sp 5		7 (3)*			
P. sp 6					5 (1)*
P. sp 7					7 (4)*
P. sp 8	1 (1)*				
Phoma glomerata	1 (1)*				
Staphylotrichum coccosporum				1 (1)*	
Talaromyces aff *helicus*		1 (1)*			2 (1)*

* Number of insect examined with fungi present

Aspergillus fumigatus Fresenius *Aspergillus terreus* Thom, two species of *Cladosporium,* five species of *Penicillium* and *Staphylotrichum coccosporum* (Meyer et Nicot) and sterile dematiaceus mycelia on seven of the ten individuals (three males and three females) - (Table 1).

All of the ten specimens of *T. infestans* (five males and five females) collected from Santa Fe province showed CFUs belong to five genera (one species of *Acremonium*, two species of *Aspergillus*, two species of *Cladosporium*, six species of *Penicillium* including *Penicillium griseofulvum* Dierkx, and one species of *Talaromyces*). The presence of a sterile dematiaceous mycelium in one individual and a sterile hyaline micelium in another individual was determined (Table 1). Colony-forming units belonged to five genera: two *Aspergillus* species, *Beauveria bassiana* (Balsamo) Vuillemin, one species of *Cladosporium,* two species of *Penicillium* and one species of *Talaromyces* on six of the ten insects analysed from Salta (four males and two females) (Table 1).

Discussion

Insect gut fungal micofloras have already been reported for several insect species such as bees (Gilliam & Prest, 1972, 1977), termites (Zoberi & Grace, 1990), lepidopterans (Ismail & Abdel-Sater, 1993), mosquitoes (Costa & Oliveira, 1998; Moore 1971; Schlein *et al*., 1985), beetles (Moore, 1971), cockroaches (Moraes *et al*., 2000) and in other triatomines (Moraes *et al*., 1998).

Moraes *et al*. (1993), Moraes *et al*. (1998), Moraes *et al*. (2000, Moraes *et al*., (2001b), reported several species of *Aspergillus* in the digestive tracts of the triatomine *Rhodnius prolixus*. In further studies Moraes *et al*. (2000, 2001b), reported *Aspergillus* (12), *Penicillium* (10) and 2 representatives of the following genera: *Cladosporium*, *Curvularia*, *Fusarium*, *Trichoderma*, *Alternaria*, *Botrytis*, *Colletotrichum*, *Paecilomyces*, *Ulocladium* and mycelia sterilia for the first paper and for the second one: *Penicillium* (19) *Aspergillus* (17) and *Acremonium* (5). New records of fungi never reported for triatomids fungal flora before are *Arhtridium phaeospermum, B. bassiana*, *Phoma glomerata, Staphillotrychum coccophilum* and *Talaromyces* aff *helicus*. Predominance of *Penicillium* and *Aspergillus* species are remarkable in our study as well as in previous references (Moraes *et al*., 2000, Moraes *et al*., 2001b,) and they are strongly represented also in the environment. Several of the fungal species we identified in this study such as *Alternaria alternata*, *A. flavus*, *B. bassiana* and *Acremonium* sp. were reported previously as pathogenic to plants and animals including insects. *Beauveria bassiana* was found for the first time in the gut fungal flora but none of the examined insects showed visible signs of infection. Moraes *et al*. (2001b) recorded a great reduction in the fungal flora population in Triatomine bugs infested with the flagellate protozoan *Trypanosoma cruzi*, but we did not detect in the present study any infesting the triatomine hosts.

Various species of fungi can be isolated from triatomines but further research is still needed to establish their possible function in insect physiology and interactions between hosts and their fungal flora.

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