SHORT COMMUNICATION

ABSTRACT

The family Simuliidae is the host of simbiontes fungi that inhabit the digestive tracts of arthropods. This paper reports the presence of fungi in *Simulium goeldii* Cerqueira & Nunes de Mello larvae in Amazonia. We observed that the larvae are a good component of aquatic systems to isolate filamentous fungi.

**Key-words:** Black fly, *Simulium*, aquatic insects, Fungi
Filamentous fungi in S. goeldii containing PDA (potato dextrose agar) culture media, to which 0.05 g per l of chloramphenicol was added. The plates were incubated at 28°C and examined every three days for 20 days. To preserve the genus of each isolate the colonies were transferred to test tubes (16 × 100 mm) containing 5 ml of PDA (2). These tubes were kept in a chamber under the same conditions of temperature (28°C) and relative humidity (80%).

To observe the macroscopic characteristics for identification of the various genera in each isolate, fragments of the colony grown in the test tubes were transplanted using a platinum loop to Petri dishes containing the media potato dextrose agar (PDA), Czapek-Dox-agar (CZ) and malt extract agar (MEA) (Difco), and were incubated at 28°C.

The cultures were identified by microscopic characteristics (sexual and asexual) using the slide culture technique and specific literature (3,8,14,20,21). Species were mounted in Amann lactophenol plus cotton blue and observed under a compound microscope.

Voucher cultures were preserved in hemolysis tubes (15 × 100 mm) with PDA under a 1-cm layer of mineral oil and incorporated in the fungus culture collection of the Instituto Nacional de Pesquisas da Amazônia (INPA) and Coleção de Culturas de Fungos do Departamento de Micologia, Instituto Oswaldo Cruz- FIOCRUZ/IOC.

A total of 50 larvae of S. goeldii were dissected, from which 36 fungal lineages were isolated. Of the isolated lineages, only 42% produced spores. The genera/species of fungi identified were: Aspergillus ochraceus Wilhelm, Penicillium bilaii Chalabuda, Penicillium chrysogenum Thom, Penicillium variabile Sopp, Paecilomyces aeruginus Samson, Beauveria bassiana (Bals.) Vuill., Metarhizium anisopliae (Metsch.) Sorokin, Trichoderma harzianum Rifié, Pestalotiopsis guepinii (Desm.) Stey, Scopulariopsis brevicaulis (Saccardo) Bainer, Acremonium sp. and Gliocadium sp. (Table 2).

The lineages that did not sporulate were inoculated in a poor culture medium, such as agar-water and agar-oats to induce the production of spores; however, sporulation was not observed. The sterile lineages were preserved in sterilized distilled water and saved for later molecular identification.

The isolated fungi in this study are widely distributed in the environment and can be isolated from soil sample and plants (2). Some of the isolated lineages have associated entomopathogenic genera/species such as M. anisopliae, Acremonium, Paecilomyces, B. bassiana and T. harzianum (2,16,17,18).

B. bassiana is frequently obtained from soil samples and insects (3), and lineages of this species are used to control populations of pest insects in the orders Coleoptera, Hemiptera, Lepidoptera, and some species of Diptera such as Musca domestica Linnaeus and Anastrepha ludens Loew (3,16,17).

| Site  | Habitat                                                      | Date            | Collector  | Longitud/ Latitud  |
|-------|--------------------------------------------------------------|-----------------|------------|--------------------|
| 1     | HW AM010 km 24, Reserva Florestal Adolpho Ducke, Igarapé Barro Branco, Manaus municipality | 28/4/2004 02/5/2004 | Fonseca, Q.R. | 02°34’S; 60°07’W |
| 2     | HW AM010, Ramal Acará, Balneário da SEDUC, Manaus municipality | 16/7/2004       | Fonseca, Q.R. | 02°56’S; 59°59’W |
| 3     | HW BR174 km 107, Igarapé da Santa Cláudia, Presidente Figueiredo municipality | 18/5/2004       | Fonseca, Q.R. | 02°02’S; 60°00’W |
| 4     | HW AM010 km 02, Ramal Baixo Rio, Igarapé Família Dó-re-mi, Rio Preto da Eva municipality | 28/9/2004       | Alencar, Y.B. | 02°42’S; 59°42’W |

HW: highway; BR: Federal highway; AM: Amazonas state highway.

Table 1. Simulium goeldii (Diptera: Simuliidae) collection sites in Amazonia.

Table 2. Frequency of fungal isolates of S. goeldii larvae (Diptera: Simuliidae) collected at different localities in Amazonia.

| Species                  | Isolated/ Frequency | Site |
|--------------------------|---------------------|------|
| Acremonium sp.           | 1 (2.8)             | 1    |
| Aspergillus ochraceus    | 1 (2.8)             | 1    |
| Beauveria bassiana       | 1 (2.8)             | 1    |
| Gliocadium sp.           | 2 (5.5)             | 3    |
| Metarhizium anisopliae   | 1 (2.8)             | 1    |
| Paecilomyces aeruginus   | 2 (5.5)             | 1    |
| Penicillium bilaii       | 1 (2.8)             | 2    |
| Penicillium chrysogenum  | 1 (2.8)             | 4    |
| Penicillium variabile    | 1 (2.8)             | 3    |
| Pestalotiopsis guepinii  | 1 (2.8)             | 2    |
| Scopulariopsis brevicaulis | 1 (2.8)         | 1    |
| Trichoderma harzianum    | 2 (5.5)             | 1    |
| Mycelia sterilia         | 21 (58.3)           | 1,2,3,4 |

Notes: Collection number refers to sites listed in Table 1.
M. anisopliae was the first species of fungus to be used in the microbial control of insects and occurs naturally in more than 300 species of different orders, including important pest insects. It is frequently used in the control of *Deois flavopicta* Stal and *Zulia enterriana* Berg (2).

Wicklow et al. (27) reported that *Acremonium* produce peptides with antimicrobial activity, having toxic properties and ability to form pores in membranes. This same study demonstrated that the antibiotic produced by *Acremonium zeae* Gams & Sumner inhibits the growth of *Aspergillus flavus* Link and *Fusarium verticillioides* Sacc. (Nirenberg). Also, this antibiotic showed activity against *Candida albicans* Sullivan and several other Gram-negative bacteria.

The fungus *A. ochraceus* is pathogenic to humans and other animals, being known to produce ochratoxina A, a toxic substance that contaminates food, especially grains such as corn and peanuts (9,15).

In assays made with insects in the family Culicidae using lineages of the fungi *Penicillium, Aspergillus* and *Trichoderma* it was observed larval mortality of nearly 70% over a period that rarely reached 72 hours (18). Several papers about *Trichoderma* have reported its potential for producing antibiotic peptides and hydrolytic enzymes; however, there is no report of the use of these metabolites to control insects (4,10,24,25).

During this study some genera/species of fungi known worldwide as important in the biological control of crop pests such as *M. anisopliae* and *B. bassiana*, were isolated from black-fly larvae. Also, from this insect fungi were isolated that are known to produce compounds of biotechnological interest, such as *T. harzianum* and *Acremonium*, but there are few studies of its potential for biological control of insect vectors of tropical diseases. Future studies on the lineages obtained need to be done to increase our knowledge of microorganisms with biotechnological potential in the Amazon region, thereby contributing to strengthening applied research in this region.

RESUMO

Ocorrência de fungos filamentosos associados a larvas de *Simulium goeldii* Cerqueira & Nunes de Mello da Amazônia Central, Brasil

A família *Simuliidae* é hospedeira de fungos simbiontes que habitam o trato digestivo de artrópodes. Este estudo reporta a presença de fungos em larvas de *Simulium goeldii* Cerqueira & Nunes de Mello da Amazônia. Foi observado que as larvas são bons componentes do sistema aquático para isolar fungos filamentosos.

Palavras-chave: Borrachudo, *Simulium*, insetos aquáticos, fungos

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