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An intensive search for promising fungal biological control agents of ticks, particularly *Rhipicephalus microplus*

Éverton K.K. Fernandes[a,b,1], Isabele C. Angelo[a], Drauzio E.N. Rangel[b,2], Thiago C. Bahiense[a,3], Áurea M.L. Moraes[c], Donald W. Roberts[b], Vânia R.E.P. Bittencourt[a,*]

[a] Curso de Pós Graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ 23890-000, Brazil
[b] Department of Biology, Utah State University, Logan, UT 84322-5305, USA
[c] Laboratório de Taxonomia, Bioquímica e Bioprospecção de Fungos, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ 21450-900, Brazil

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ABSTRACT

Entomopathogenic fungi have been investigated worldwide as promising biological control agents of the cattle tick *Rhipicephalus microplus*. The current study evaluates the virulence of several fungal isolates to *R. microplus* larva in the laboratory as part of an effort to identify isolates with promise for effective biocontrol of *R. microplus* in the field. Sixty fungal isolates, encompassing 5 *Beauveria* spp. and 1 *Engyodontium albus* (=*Beauveria alba*), were included in this study. In addition to bioassays, the isolates were characterized morphologically and investigated as to their potential for conidial mass production. These findings were correlated with previous reports on the same fungal isolates of their natural UV-B tolerance (Fernandes et al., 2007), thermotolerance and cold activity (Fernandes et al., 2008), and genotypes (Fernandes et al., 2009). *R. microplus* larvae obtained from artificially infested calves were less susceptible to *Beauveria bassiana* infection than ticks acquired from naturally infested cattle from a different location. Isolates CG 464, CG 500 and CG 206 were among the most virulent *Beauveria* isolates tested in this study. All fungal isolates presented morphological features consistent with their species descriptions. Of the 53 *B. bassiana* isolates, five (CG 481, CG 484, CG 206, CG 235 and CG 487) had characteristics that qualified them as promising candidates for biological control agents of *R. microplus*, viz., mean LC_{50} between 10^7 and 10^8 conidia ml^{-1}; produced 5000 conidia or more on 60 mm^2 surface area of PDAY medium; and, in comparison to untreated (control) conidia, had the best conidial tolerances to UV-B (7.04 kJ m^{-2}) and heat (45 °C, 2 h) of 50% or higher, and conidial cold (5 °C, 15 d) activity (mycelial growth) higher than 60%. The current study of 60 *Beauveria* spp. isolates, therefore, singles out a few (five) with high potential for controlling ticks under field conditions.

1. Introduction

The tick *Rhipicephalus* (*Boophilus*) *microplus* Canestrini, 1887 (Acari: Ixodida) (Murrell and Barker, 2003), formerly *Boophilus microplus*, is one of the most important bovine ectoparasites in Brazil and several other countries worldwide. Economic losses to the cattle industry in Brazil alone are estimated at two billion dollars per year (Grisi et al., 2002). These economic losses include costs associated with the use of chemical acaricides for tick control. The continual use of these chemicals, however, has many negative

* Corresponding author at: Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ 23890-000, Brazil. Tel.: +55 21 2682 1617; fax: +55 21 2682 1617.
E-mail address: vaniabit@ufrrj.br (V.R.E.P. Bittencourt).
[1] Current address: Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO 74605-050, Brazil.
[2] Current address: Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, SP 12244-000, Brazil.
[3] Current address: Instituto de Ciências da Saúde, Departamento de Biointeração, Universidade Federal da Bahia, Salvador, BA 40110-100, Brazil.

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side effects; including the development of chemical resistance in tick populations, as well as food and environmental contamination if the products are improperly used. Public concerns about the environmental impacts and safety to vertebrates of widespread chemical acaricide use are driving research towards alternative, sustainable methods for tick control, including biological control (Chandler et al., 2000).

Entomopathogenic fungi (EPF) are natural enemies of arthropods, and they have been investigated worldwide as promising biological control agents of ticks (Chandler et al., 2000; Fernandes and Bittencourt, 2008; Samish and Rehacek, 1999). *Beauveria* (Balsamo) Vuillemin (Ascomycota: Hypocreales) is one of the EPF groups most commonly studied; primarily due to their cosmopolitan geographical distribution, wide host range, and capacity to cause enzootic and epizootic outbreaks in several arthropod pests (Alves, 1998; Roberts and Campbell, 1977).

Due to environmental factors, especially the exposure of conidia to strong solar irradiation, reduced viability and/or conidia germination delay is fully expected to reduce the bioinsecticidal efficacy of fungal inocula in field situations (Braga et al., 2002; Fargues et al., 1997). In addition, conidia in the environment also are exposed to indirect solar effects, such as heat and desiccation (Luz and Fargues, 1997; Magalhães and Boucias, 2004; Rangel et al., 2005). Through selection of the isolates most tolerant to UV-B radiation as well as incorporating UV protectants in formulations, it may be possible to significantly prolong the persistence and increase efficacy of these fungi in highly insolated habitats (Fargues et al., 1996). Also, effective use of insect pathogens within integrated tick management programs necessitates the selection of fungal pathogens tolerant to the temperature range found in the host arthropod's ecosystem, including on the skin of these warm-blooded animals.

The current study investigates the virulence of 60 *Beauveria*-like fungal isolates to *R. microplus* larva (in a search for isolates with high potential for biological control of this tick species). Bioassay ticks originated from two different locations, and from either artificially or naturally infested cattle. In addition, the fungal isolates were characterized morphologically; and the isolates with the highest potential for conidial mass production identified. The findings were compared with previous studies of the same isolates which evaluated UV-B tolerance (Fernandes et al., 2007), elevated heat tolerance and cold activity (Fernandes et al., 2008), and their genotypes (Fernandes et al., 2009). Comparisons of these findings allowed the selection of a short list of isolates most promising for further investigation as tick biocontrol agents.

2. Materials and methods

2.1. Fungal isolates

Fifty-three *Beauveria bassiana* isolates were included in this study: 49 originating from different regions of Brazil and 4 from USA. In addition, 6 isolates of other *Beauveria* spp. (3 *Beauveria amorpha*, 1 *Beauveria brongniartii*, 1 *Beauveria velata* and 1 *Beauveria vermiconia*), and 1 isolate of *Engyodontium albus* (= *B. alba*) were included. The fungal isolates were originally from Acari, six orders of insects, or soil. This study emphasized Brazilian *B. bassiana* isolates; with isolates of other *Beauveria* species or from other geographic origins being used primarily as general references. The fungal isolates investigated in the current study were known to have considerable genotypic variation (Fernandes et al., 2009). The designation of the isolates, the culture collections from which they were obtained, their geographical origins, and hosts or substrates from which they were isolated are available at Fernandes et al. (2007, 2008, 2009).

2.2. Bioassays

2.2.1. *R. microplus* larvae from eggs from engorged females collected on artificially infested calves (Group A)

Engorged females of *R. microplus* were manually collected directly from naturally tick-infested cattle at the Universidade Federal Rural do Rio de Janeiro (UFRRJ) dairy farm, Seropédica, Rio de Janeiro State, Brazil. Ticks were surface sterilized by immersion in a 1% sodium hypochlorite solution for 3 min, rinsed with sterile distilled water and dried with sterile tissue paper. The engorged females were held in polystyrene Petri plates (95 mm × 15 mm, BD Falcon®, São Paulo, SP, Brazil) incubated in the dark at 27 ± 1 °C and ≥80% relative humidity (RH) for oviposition. Eggs were separated in aliquots of 100 mg (approximately 2000 eggs) and incubated at the same temperature and RH conditions to allow them to hatch. Four calves were held in individual pens at the UFRRJ W.O. Neitz Station for Parasitology Reseach, and each calf was artificially infested once with 6000 larvae. Twenty-one days after infestation, engorged females were manually collected directly from the calves and from the floor of each pen. Ticks were taken to the laboratory for cuticle antisepsis and oviposition as described above. Ten days after the beginning of oviposition, the eggs were divided into 50 mg aliquots (approximately 1000 eggs) and placed in glass test tubes (150 mm × 15 mm, Pyrex®, São Paulo, SP, Brazil). The test tubes were sealed with hydrophilic cotton plugs and incubated at 27 ± 1 °C and RH ≥ 80%. Since *R. microplus* larvae have strong negative geotropism and positive phototropism, the tubes were held vertically with the cotton plugs down (in the shadows), and the glass end in the light. The bioassays were carried out 10 days after total hatch. The tubes that did not have complete hatch were discarded to ensure that each test tube had approximately 1000 live *R. microplus* larvae.

2.2.2. *R. microplus* larvae from eggs from engorged females collected on naturally infested cattle (Group B)

Five *B. bassiana* isolates from among the 60 isolates previously tested against Group A ticks, due to their low virulence, were chosen to be tested on *R. microplus* larvae obtained from engorged females collected directly from naturally tick-infested cattle (Group B) on a private farm located on highway Presidente Dutra, Km 201, in Seropédica, RJ. This farm is approximately 10 km from the UFRRJ dairy farm where Group-A ticks were collected. Engorged females were surface sterilized and held in the