III CONGRESSO LATINOAMERICANO DE ACAROLOGIA E VI SIMPÓSIO BRASILEIRO DE ACAROLOGIA
29 DE JULHO A 02 DE AGOSTO DE 2018 - PIRENÓPOLIS, GOIÁS, BRASIL
ISBN: 978-85-66836-21-9

EVALUATION OF MSN2 TRANSCRIPTION FACTOR IN THE INFECTION PROCESS OF *Beauveria bassiana* s.l AGAINST *Rhipicephalus microplus*

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Beauveria bassiana s.l. is one of the most investigated fungi for tick control. The Msn2 is a transcription factor associated to fungal growth, conidiogenesis, stress-response and virulence of *B. bassiana* against *Galleria mellonella* and *Spodoptera litura*, both lepidopteran insects. In the current study we evaluated the interference of Msn2 transcription factor in the infection of *B. bassiana* against *Rhipicephalus microplus* (Acari, Ixodidae). A Msn2 knockout-mutant strain of *B. bassiana* (BbΔmsn2) was compared to its wild type (BbWT) and the complement mutant (Bbmsn2/BbΔmsn2). Engorged females were treated topically with 2 µL of conidial suspension [1.0×10^8 conidia mL^{-1}] of each strain. Ticks were incubated at 25 ± 1 °C and relative humidity ≥ 98% for 48, 72, 96 or 120 h; each fungal-treated engorged female was fixed in a mixture of 2% (v/v) glutaraldehyde, 2% (v/v) paraformaldehyde in 0.1 M sodium cacodylate buffer solution, pH 7.2, for 10 days. The samples were dried, placed on a stub, coated with gold and analyzed by scanning electron microscopy (SEM). Treated ticks incubated for 120 h were also cut longitudinally, dehydrated and embedded in resin, 1:2 in relation to ethanol 100%, and then the resin proportion was increased gradually; finally, the samples were embedded in pure resin plus hardener solution. Sections of 3 µm were made using a microtome; the sections were then stained with periodic acid-Schiff and Green light, and analyzed with a light microscope. Scanning electron micrographs of all strains showed a large number of conidia adhered to the tick cuticle. Germ tubes from germinating conidia were seen in the shortest incubation times investigated, 48 and 72 h; larger germ tubes were seen at 96 h. Histological sections of ticks treated with Bbmsn2/BbΔmsn2 and incubated for 120 h showed fungal penetration through the cuticle, and it reached the tick interior. Hyphae of BbΔmsn2, however, did not trespass the tick cuticle after 120 h incubation. Our partial results indicate that the absence of the Msn2 transcription factor did not impair germination of *B. bassiana* s.l on *R. microplus* cuticle but delayed the fungal penetration through the tick cuticle. Histological sections of treated ticks incubated for extra periods are being investigated to elucidate if Msn2 transcription factor interfere in the initial steps of *B. bassiana* infection against *R. microplus*.

Keywords: cattle tick, biocontrol, histology, scanning electron microscopy.
Financial support: Capes, CNPq.