Secretome analysis of Metarhizium anisopliae reveals the expression of stress and infection related proteins using an in vitro model of cattle tick infection

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The fungus *Metarhizium anisopliae* is used to control several arthropod pests worldwide, including the cattle tick *Rhipicephalus microplus*. Understanding the molecular events implicated in the tick infection, including stress-related response is crucial to improve the biocontrol. Accordingly, a secretomic approach was applied to identify differentially secreted proteins expressed by two strains of M. anisopliae, one virulent (strain E6) and another non-virulent (NE) to cattle tick. Those proteins were identified in two important infection stages, the spore surface and secreted proteins during the mycelial stage induced by host cuticle triggering the molecular machinery of infection. The proteomic strategy applied was the MudPIT followed by the use of different in silico analyses, in order to: identify and quantify differentially expressed proteins, presence of hypothetical proteins and reannotation, identify conserved domains, subcellular localization and gene ontology. The preliminary results revealed 131 proteins identified being 55 unique to the NE strain, 21 unique to the E6 strain, and 55 differentially expressed found in both. Using BlastP, 34 hypothetical proteins were found within all 131, 4 of which could be re-annotated. In the mycelium secretome, 291 proteins were identified: 6 unique to the NE strain, 199 unique to the E6 strain, and 86 differentially expressed. Using BlastP, 72 hypothetical proteins were found within all 291, 8 of which could be re-annotated and 25 had at least one conserved domain. The analysis of signal-peptides was carried through 5 online programs (Phobius, PrediSi, SignalP 5.0, TargetP 2.0 and WoLF PSORT) and 48.19% and 75.28% of the identified proteins in the secretomes of the spore and mycelium surfaces, respectively, presented a conserved secretion signal-peptide detected in at least three of the five programs, attesting the expected enrichment of secreted proteins in the samples. The OmicsBox-Blast2GO was used to annotate protein ontology regarding its predicted biological processes and molecular function. Accordingly, 18 proteins were found related to stress-response processes in the spore sample and 53 in the mycelium sample that might be linked to fungal protection in the environment and potentially during host infection, including known host immunomodulation proteins. Among those, tyrosinase 2, GMC oxidoreductase and subtilisin-like serine proteases PR1C, PR1G, PR1J and PR1K can be cited. In conclusion, the preliminary results presented in this work show a substantial list of proteins that may explain how several proteins could lead to fungal virulence of cattle tick and stress response in spore and during the infection.

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