

TITLE: FUNCTIONAL ANALYSIS OF A CHITINASE GENE ACQUIRED THROUGH HORIZONTAL GENE TRANSFER IN THE ENTOMOPATHOGEN *Metarhizium anisopliae*

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ABSTRACT:

The *Metarhizium* genus consists of cosmopolitan fungi that infect a wide range of arthropod hosts. The infection is a complex process enclosing several steps, typically culminating in host death. During infection, *Metarhizium spp.* form different infection-specialized cellular structures and secrete a set of hydrolytic enzymes that helps to breach the chitin-rich host cuticle and to access the host hemolymph. Chitinases are amongst these secreted enzymes and catalyze the hydrolysis of β -1,4 glycosidic bonds between GlcNAc monomers in chitin. In fungi, chitinases have, nutritional importance and exhibit morphogenic and autolytic functions, acting on different processes through development and life cycle maintenance. Fungal glycoside hydrolase family 18 (GH18) contains enzymes with chitinase and endo- β -D-N-acetylglucosaminidases (ENGases) activities. We described from *M. anisopliae* E6 strain genome analysis twenty-four putative genes containing the GH18 domain, which were classified into five subgroups. Nine genes were assigned to subgroup A, seven to B, four to C, one gene to D and three to a subgroup containing only ENGases. The importance and function of *chimaD1* gene, horizontally acquired GH18 subgroup D chitinase from bacteria remains unknown in *M. anisopliae*. Here we evaluate the function of the *chimaD1* gene in the fungus life cycle, as well as to exploit its phylogenetic distribution and conservation in filamentous fungi. The *chimaD1*-disrupted strain and a complemented strain have been constructed. These strains do not have any hypha, blastospore nor appressorium morphology alterations. Alterations in sensibility to stressor agents (such as CaCl₂, Calcofluor White, Congo Red, H₂O₂, KCl, NaCl) were not detected. The constructed *chimaD1*-disrupted strain displayed higher virulence towards mealworm larvae (*Tenebrio molitor*) and cattle tick (*Rhipicephalus microplus*) when compared to wild type strain. Further, the phylogenetic analysis have improved previous results showing that this chitinase gene was horizontal acquired by a Hypocreales order ancestor. The higher virulence exhibited by *chimaD1*-disrupted strain was unexpected, we hypothesize that this phenotype can be linked with the unbalance of other hydrolytic enzymes and a putative compensatory effect. This results reinforce the complexity of chitinolytic system in filamentous fungi.

KEYWORDS: *Metarhizium*, chitinase, knockout strain, horizontal gene transfer.

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