

# THE ORIGIN AND ARCHITECTURE OF *TRICHODERMA* HYDROPHOBOME

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## INTRODUCTION

Higher filamentous fungi (subkingdom Dikarya) are commonly known as moulds, mushrooms or toadstools. Their vegetative body, *mycelium*, can be described as an apically growing branching tube (the *hypha*), which in macroscopic species aggregates into large structures such as fruiting bodies. To facilitate their reproduction, dispersal, and survival in unfavourable conditions these organisms (fungi *sensu stricto*) form various spores that can be produced on a diversity of hyphal structures such as conidiophores or sporocarps. Vegetative hyphae usually grow inside their food and feed by secreting digestive enzymes into their environment and absorbing dissolved small molecules. Therefore, a hyphal organisation of the body provides high surface area-to-volume ratio that is a key adaptation for the efficient extraction of nutrients while growing on or in solid substrates or germinating as spores in a liquid. Hyphae are specifically adapted for efficient attachment to a diversity of solid surfaces; they can also invade substrates and tissues due to large penetrative mechanical forces that they can also exert because of efficient attachment abilities. The development of many fungi includes interchanges between penetration of tissues/substrates for nutrition and growing out of them for dispersal. Undoubtedly, fungi have unique molecular adaptations that emerged in the course of the evolution of their distinctive life style. Hydrophobins are the small amphiphilic surface active proteins containing eight conserved cysteine residues that are produced only by filamentous fungi<sup>1-3</sup>. Some mushrooms (Basidiomycota) have a rich arsenal of hydrophobins that are required for the formation of their fruiting bodies and spore distribution, while the rest of them and the majority of Ascomycota have only a few hydrophobin-encoding genes. In this respect, the mycoparasitic genus *Trichoderma* (Hypocreales, Pezizomycotina), which also includes several cosmopolitan generalist species with high environmental opportunistic potential<sup>4</sup>, is as an exception as genomes of these fungi have expanded the number of hydrophobins. Previous studies have revealed that most of *Trichoderma* hydrophobins are the orphan genes that have no homologues in related organisms, while other Pezizomycotina fungi share their hydrophobins. The first genome-wide studies of *Trichoderma* provided the evidence for the operation of purifying natural selection pressure for *Trichoderma* hydrophobins that results in “birth-and-death” evolution of these proteins<sup>5</sup>.

In this study, we investigate the evolution of hydrophobin-encoding genes in the genus *Trichoderma* and compare it to other fungi from the order Hypocreales and beyond.

## METHODS

First, the genomes of 11 *Trichoderma* spp. and 12 genomes of other Hypocreales fungi were mined for hydrophobin-encoding genes. Then, the resulting library amino acid sequences (~140 OTUs)

was subjected to sequence alignment and phylogenetic analysis by Bayesian methods. Best fit substitution model was selected based on BIC criterion using Smart Model Selection tool. Evolutionary analysis using two independent runs of 5 million MCMC generations resulted in reliable diagnostic parameters estimated based on potential scale reduction factor, effective sample size and average standard deviation of split frequencies. The hypothesis of gene loss, gene duplication, and horizontal gene transfer (HGT) events were tested using NOTUNG 2.9, HGT was also verified by TRex. The operation of positive selection pressure was tested using the  $K_a/K_s$  ratio and Tajima  $D$ -test as implemented in DnaSP v5. The expression analysis for hydrophobin-encoding genes has been performed by mining available transcriptomic databases for *T. reesei*. As the former species has the smallest hydrophobome (HFBome) in *Trichoderma*, an *in vitro* analysis of the expression of hydrophobin-encoding genes was performed for *T. virens*, *T. harzianum* and *T. guizhouense* that have the largest *Trichoderma* HFBomes to date. Physical-chemical properties of individual hydrophobins have been calculated based on such parameters as the surface hydrophobicity, hydropathy plots, pI, and others.

## RESULTS AND DISCUSSION

This study provides the exhaustive evolutionary survey of hydrophobin-encoding genes and respective proteins in *Trichoderma* and other Hypocreales fungi. Phylogenetic analysis revealed at least 14 monophyletic clades containing active individual hydrophobin-encoding genes in *Trichoderma*. In several cases, the events of gene loss and duplication have been confirmed statistically. The analysis of the selection pressure revealed a highly heterogeneous pattern when individual genes in some species appeared to be under the strong pressure of either purifying or positive selection while the respective homologous genes evolved neutrally in the other taxa.

## CONCLUSION

Our study demonstrates that the expansion of HFBome in the genus *Trichoderma* and its unique architecture are closely linked to the ecological adaptations of this genus. The phylogenomic analysis allowed to differentiate evolutionary old (plesiomorphic) from the newly emerged (apomorphic) hydrophobins. We show that the evolution of the entire HFBome most likely contributes to the fitness of an individual species, while individual genes may emerge and get lost relatively quickly. Thus, our study points to the importance of the expression regulation of hydrophobins and the important role of hydrophobin-hydrophobin interactions for the survival of *Trichoderma*. We also propose a model that explains the role of HFBome in the emergence of superial environmental opprtunism of this fungus.

In this work, the nomenclature of class II hydrophobins of Ascomycota fungi has been developed.

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