

<b>Dissertation Title</b>	Characterization of <i>LtLysMI</i> Gene in <i>Lasiodiplodia theobromae</i> and Taxonomy and Phylogeny of Selected Ascomycetes
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## **ABSTRACT**

The kingdom of fungi is one of the largest and the most diverse groups, with approximately 2.2–3.8 million introduced species and new species are being added every day to its roster. Due to its cosmopolitan distribution, many species are yet to be discovered in the fungal kingdom from their poorly explored habitats and a variety of biodiversity hotspots. The phylum Ascomycota, the largest in the fungal kingdom, consists about 19 accepted classes consisting of over 64000 different species. Among them, Dothideomycetes and Sordariomycetes are the most phylogenetically diverse classes with species from variety of lifestyles such as pathogenic, endophytic, saprobic and many more to discovered from terrestrial and aquatic habitats globally. Even though the past few decades have seen a substantial increase in research related to fungi due to their agricultural, biotechnological and pharmaceutical value, many species in both Dothideomycetes and Sordariomycetes are still very poorly understood and not studied comprehensively. These gaps in knowledge must be filled with more organized and wide-ranging studies to understand their diversity and potential.

The pathogenic potential of the fungi has been an important area of study due to the devastating economic losses that occur in food and crop production. Identification and characterization of these pathogens and understanding their disease causing potential and underlying mechanisms have become important step in disease

management in the recent years. Genomic studies are an important study area in understanding the pathogenic potential of fungi. Genome editing using the CRISPR/Cas9 system is one such approach used in the recent years. Even though this technology is fairly recent, many pathogens of economically important crops have been subjected to in-depth studies using this molecular tool. Herein, I describe the importance of this molecular tool in studying phytopathogenic fungal genomes and how they can be further extended for studies related to pathogenesis process and identification of key genes involved in the process. I also describe how this relatively cheaper, easier to use, and efficient genetic tool has opened many interesting applications for the future of genomic studies and pathogen control.

Botryosphaeriaceae, an important member of the Dothideomycetes, is a geographically diverse fungal group associated with a wide range of economically and environmentally important woody crops. Grape, one of the most economically important crops worldwide, is susceptible to pathogens from Botryosphaeriaceae. The last few decades have seen a substantial increase in comprehensive pathogen identification, but less studies were conducted to understand the molecular aspects related to the pathogenesis. Due to the increase in genomic and transcriptomic studies focused on botryosphaeriaceous pathogens in the recent years, the knowledge related to the genetic basis of pathogenicity has advanced significantly. In China, grape infecting botryosphaeriaceous pathogens such as *Botryosphaeria dothidea*, *Lasiodiplodia theobromae* and *Neofussicoccum parvum* have been reported to cause significant damage and economic losses. Among them, *Lasiodiplodia theobromae* has been recorded as the most virulent. The various comparative genome and transcriptome analyses have provided an insight into what type of genetic background allows these fungi to act as pathogens. Plants have a very sophisticated two-line defense system to face pathogen attacks. Pathogens have the ability to overcome these defenses by secreting proteins known as effectors. LysM motif containing effectors are one such group of secretory proteins that allow the pathogen to overcome plant defense systems and the colonization of the host. In the current study, I am focusing on one of the LysM

effectors, *LtLysM1*, of *Lasiodiplodia theobromae* capable of circumventing the plant defenses and aid in pathogenicity. *LtLysM1* was confirmed as a secreted protein using the yeast signal peptide trap assay. The gene was cloned and over-expressed in *Lasiodiplodia theobromae*, resulting in an increase in the pathogenicity on *Vitis vinifera* and reduced pathogenicity was observed when RNA-interference approach. This indicated that the secreted *LtLysM1* function as a critical virulence factor during the symptom development in woody plants.

The identification of fungal species has been an interesting and a highly debated topic due to their phenotypic plasticity and polyphyletic nature. Introducing species using both morphological and molecular data has been implemented in the recent years to provide intricate details on a large number of species. In this study, both of these approaches are used to in the investigation of ascomycetes. Fungal species isolated from various hosts from China and Italy were studied.

In addition to studying fungi collected from specific hosts or regions, this study was further expanded to include various new host records and new species from miscellaneous specimens collected from Italy. Species identification with phylogenetic approaches such as Maximum parsimony, Maximum likelihood and Bayesian posterior probabilities using various combinations of gene regions were used in these studies. Appropriate illustrations and descriptions were provided as evidence for accurate identification.

**Keywords:** LysM, Effectors, Grape, Pathogenicity, Fungal genomes, CRISPR/Cas9