Biotechnology of Fungal Genes

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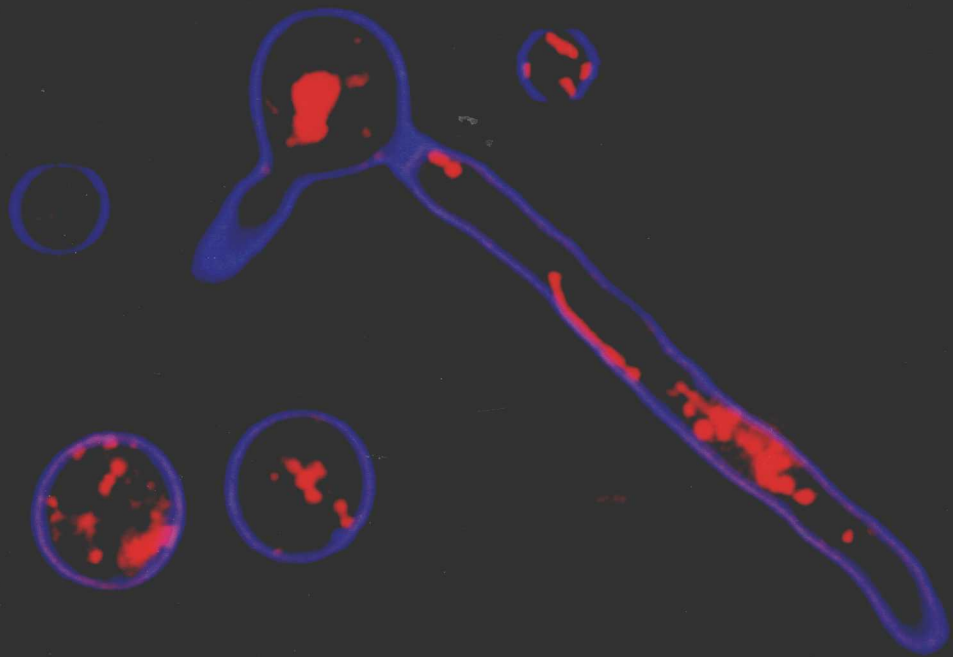

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Biotechnology of Fungal Genes

Foreword

Biotechnology simply defined as the application of living organisms and their components to industrial products and processes is not an industry in itself, but an important technology that will have a large impact on many different industrial sectors in the future. For biotechnological processes genes are the basic functional unit, which encodes instructions on how to synthesize proteins that are carried on chromosomes in all kind of organisms. Genetic research has provided important knowledge about genes, heredity, genetic mechanisms, metabolism, physiology and development in micro- and microorganisms. Several new technologies have attracted tremendous interests among biologists to study the physical and functional aspects of genes. Among microorganisms fungi exhibit a wide range of biosynthetic and bio-degradative activities. Man has been familiar with fungi since times immemorial, for example prehistoric people used yeast cells to raise bread dough and to ferment alcoholic beverages, to make cheeses and yogurts. In the past few decades, filamentous fungi have grown for commercial importance not only in the food industry but also as sources of pharmaceutical agents for the treatment of infectious and metabolic diseases and of proteins and enzymes used to process foods, fortify detergents and to perform biotransformation. The commercial impact of molds is also measured on a negative scale since some of these organisms are significant as pathogens of crop plants, animals and humans, agents of food spoilage, and sources of toxic compounds.

Recent advances in the molecular genetics of filamentous fungi are finding increased application in the pharmaceutical, agricultural and enzyme industries, and this trend promises to continue as the genomics of fungi is explored and new techniques to speed genetic manipulation become available. *Biotechnology of Fungal Genes* is an excellent collection of reviews from many researchers across the world. The main focus of the book is to give an overview of knowledge on fungal genes. It explores fungal pathogenesis, identification of involvement of fungal pathogenicity genes and enhancement of fungal resistance genes; discusses the role of fungal

genes in industrial and agricultural applications through the synthesis of carotenoids, enzymes, antibiotics, biopesticides; and focused on physiology and molecular biology of fungi. This book has fully updated information to reflect the many exciting developments in the field; notably, those relating to the application of fungal molecular genetics. This book would be very useful for readers of diverse disciplines including mycologists, plant pathologists, mycotechnologists, botanists, pharmacologists, biotechnologists, environmentalists and medical mycologists.

I wish a grand success to this endeavor of Dr. Vijai Kumar Gupta, National University of Ireland. I am sure that it will fulfill the need of numerous researchers, teachers and will be useful contribution to the science. The researchers have attempted to communicate their significant observations and ideas to the scientific community. I believe that the book will expose student community also to new developments in the fungal research.

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Preface

Fungal Biotechnology offers the newest developments from the frontiers of fungal biochemical and molecular processes and industrial and semi-industrial applications of fungi. Biotechnology of Fungal Genes is a comprehensive, balanced introduction of the biology, biotechnological applications and medical significance of fungal genes. Since the introduction of technology which enables these organisms to be genetically engineered, the practical applications of fungi have increased more dramatically. Fungi now play a more important role in the manufacture of a wide range of products by fermentation, in agriculture through their use as pest and pathogen control agents and as growth enhancers, in environmental management and in the food industry. This book highlights the need for the integration of a number of scientific disciplines and technologies in modern fungal biotechnology and reigns as the top source on current molecular, biochemical, and medical technologies and commercial usages for fungi representing a broad international background. Recent advances in the molecular genetics of filamentous fungi are finding increased application in the pharmaceutical, agricultural, and enzyme industries, and this trend promises to continue as the genomics of fungi is explored and new techniques to speed genetic manipulation become available. This edited volume focuses on the filamentous fungi and highlights the advances of the past decade, both in methodology and in the understanding of genomic organization and regulation of gene and pathway expression. The approach and techniques of molecular biology enable us to ask and answer fundamental questions about many aspects of fungal biology, and open the way to the directed manipulation of fungal genetics.

Moreover, this book provides rapid development and advancement of fungal genes and the ways in which these are being exploited in species of economic importance either in biotechnology or as biochemically. Although it is particularly suitable for postgraduate students and research workers, this book will also be of interest to undergraduate students who require an

overview of the traditional and more recent practical applications of fungal genes and insight into potential areas of their future use.

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Contents

CHAPTER 1

Identification of Fungal Pathogenicity Genes by *Agrobacterium tumefaciens*-Mediated Transformation

Karunakaran Maruthachalam, Junhyun Jeon, Yong-Hwan Lee and Krishna V. Subbarao

Abstract

The fungal genomics field over the past five years has exploited *Agrobacterium tumefaciens*-mediated transformation (ATMT) for targeted and random mutagenesis to determine the functions of individual genes. Currently, the technique has become routine for genetic manipulation of a wide range of fungal species. Because of the higher transformation efficiency, ease of selecting starting materials and generating a large number of stable transformants within a few weeks, the number of fungi transformed by ATMT has steadily grown since 2005. This chapter provides an overview of ATMT method for possible functional characterization of pathogenicity genes, mainly focusing on two phytopathogenic fungal species, *Magnaporthe oryzae* and *Verticillium dahliae*.

INTRODUCTION

Phytopathogenic fungi cause many of the world's most notorious plant diseases and damage crops worth billions of dollars annually (Strange and Scott 2005, Buckley 2005). The losses caused by fungi could be either from reduction in yield or quality of the produce and this entirely depends on the fungus and crop combination and the nature of this interaction. In general, the biology, epidemiology and host-pathogen interaction

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have been historically studied, and information at the molecular level underpinning the biology and the fungal interactions with their host are becoming available only recently. Understanding the genetic basis of the fungus-host interactions and identification of specific genes involved in fungal pathogenicity is an ongoing endeavor because of the complexity of genes involved in fungal infection and disease development. In addition, the disease development requires coordinated regulation of gene expression and interaction between thousands of genes within the fungal genome making it even more challenging to understand the fungal pathogenicity (Schafer 1994, Oliver and Osbourn 1995, Hamer and Holden 1997, Knogge 1998). Our current understanding of the nature of fungus–host interactions has been derived from genetic manipulation of individual genes and gene products through the construction of null mutants, gene expression analysis and complete gene characterization.

With the advancements in whole genome sequencing technologies and the next generation high-throughput sequencing (NGS) technologies such as 454 genome sequencer FLX system (Roche Applied Science, IN, USA) and Illumina Genome Analyzer (Illumina Inc. CA, USA), there has been a rapid increase in the number of fungal genomes available. Availability of sequenced genomes has provided a unique opportunity to conduct comparative genomic studies to understand the different life styles of pathogens, their biology and evolutionary mechanisms (Shendure and Ji 2008, Nowrousia et al. 2010).

The next generation sequencing technologies have also led to the availability of genomes of a large number of agriculturally important fungi, facilitating the study of plant pathogens at the genomic scale. In addition, to translate all the structural genomics data (nucleotide sequences) of the different fungal pathogens into functional genomics data (determinining the gene function of each of the ~10,000 genes), requires robust, high-throughput transformation technique to employ into both forward and reverse genetics approaches. Once novel/virulence-associated genes are identified using the comparative genomics approach and subsequently disrupt the gene of interest from the fungus to check the phenotypes to determine whether it has any effect on virulence such as reduced or loss of disease symptoms, etc., then genetic transformation method is the potential tool for functional genomics. In some cases, the availability of the genomic sequences for both the fungus and its host plants provide a unique opportunity for the parallel study of host-pathogen interaction from both organisms using functional genomics (Jeon et al. 2007).

Transformation technology has been the basic research tool employed in the study of fungal genes at the molecular level. Transformation techniques enable stable integration of foreign DNA into fungal genome mainly

based on homologous recombination to achieve gene disruption and gene replacement with greater precision and efficiency. In addition, utilization of transformation methods for molecular characterization of genes and availability of classical genetics studies placed many species of ascomycetes including *Neurospora crassa*, *Aspergillus nidulans* and *Magnaporthe oryzae* as model organisms. Several transformation methods such as protoplast with polyethylene glycol (PEG) (Meyer et al. 2003), electroporation (Robinson and Sharon 1999) and restriction enzyme-mediated integration (REMI) (Tanaka et al. 1999, Balhadere et al. 1999, Redman et al. 1999, Thon et al. 2000) are being employed to understand the genetic basis of pathogenicity of fungal plant pathogens (Olmedo-Monfil et al. 2004, Michielse et al. 2005). In contrast to other insertional mutagenesis techniques such as REMI, *Agrobacterium tumefaciens*-mediated transformation (ATMT) does not require protoplasts and has a choice of starting materials such as conidia, hyphae and blocks of mycelia from fruiting body (Chen et al. 2000, Stone et al. 2000, Amey et al. 2002, Meyer et al. 2003, Michielse et al. 2005) to choose for transformation. In addition, ATMT method has higher transformation efficiency than other transformation techniques described so far.

Agrobacterium tumefaciens-mediated transformation has long been used to produce transgenic plants. An array of transgenic plants have been generated in *Arabidopsis thaliana* (Ostergaard and Yanofsky 2004) as also in many agriculturally important crops including rice (Hirochika et al. 2004) for functional genomic studies. With the demonstration of the expansion of host range of *A. tumefaciens* to include the budding yeast, *Saccharomyces cerevisiae* to create random insertional mutagenesis (Piers 1996), the utility of this technique was subsequently extended to other filamentous fungi including many phytopathogenic fungi (Bundock et al. 1995, de Groot et al. 1998, Chen et al. 2000, Rho et al. 2001, Mullins et al. 2001, Michielse et al. 2005). ATMT also has been widely used for targeted mutagenesis in fungi (Michielse et al. 2005, Bhadauria et al. 2009). Furthermore, with targeted gene deletion experiments, high frequency of homologous recombination was produced, indicating the effectiveness of ATMT for targeted mutagenesis (Michielse et al. 2005, Bhadauria et al. 2009).

Verticillium spp. cause Verticillium wilt in many economically important crops worldwide and cause billions of dollars crop losses annually. So far more than 400 plant species have been reported as hosts for this *Verticillium* spp. In *Verticillium*, conidia or microsclerotia germinate and initiate the Verticillium wilt disease cycle. In the absence of suitable environment or host, the microsclerotia can stay in the soil for up to 14 years (Pegg and Brady 2001). When environmental conditions are favorable, the microsclerotia germinate and produce hyphae that colonize the host roots that subsequently leads to symptom development (Fig. 1).