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The aspergilli are a fascinating group of fungi exhibiting immense ecological and metabolic diversity. These include notorious pathogens such as *Aspergillus flavus*, which produces aflatoxin, one of the most potent, naturally occurring, compounds known to man. Conversely, also included are other fungi, such as *A. oryzae*, involved in the industrial production of soy sauce and sake or *A. niger* used for the production of citric acid and enzymes such as glucose oxidase and lysozyme. Such is the interest in *Aspergillus* that, to date, the sequences of fifteen different *Aspergillus* genomes have been determined providing scientists with an exciting resource to improve the understanding of *Aspergillus* molecular genomics and act as a spring board for mining for new metabolites and novel genes of industrial or medical importance.

In this book leading *Aspergillus* researchers review and summarise the most important aspects of *Aspergillus* molecular biology and genomics. The book opens with a fascinating overview of the genus *Aspergillus*. This is followed by in-depth reviews of the *Aspergillus* molecular systematics, comparative genomics, bioinformatics and systems biology of *Aspergillus*, transcriptional regulation, genetics and genomics of sexual development of *A. nidulans*, genomics and secondary metabolism, ecology, development and gene regulation in *A. flavus*, functional systems biology, and novel industrial applications of *A. oryzae* genomics.

**Chapter 1 An Overview of the Genus *Aspergillus***

**J. W. Bennett**

*Aspergillus* is the name used for a genus of molds that reproduce only by asexual means. The morphology of the conidiophore, the structure that bears asexual spores, is the most important taxonomic character used in *Aspergillus* taxonomy. *Aspergillus* species are common and widespread. They are among the most successful groups of molds with important roles in natural ecosystems and the human economy. Among scientists working on *Aspergillus*, there is a continuing fascination with their biotechnological potential. In addition to producing numerous useful extracellular enzymes and organic acids, these molds also produce secondary metabolites of importance in biotechnology. Some *Aspergillus* species function as plant and/or animal pathogens. Aspergillosis is the name given to all animal diseases caused by growth of any

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member of the genus on a living host. Immunosuppression is generally a pre-requisite for systemic *Aspergillus* infections in humans. The incidence of systemic aspergillosis, the most serious form, is on the rise and imposes an increasing medical burden upon hospitals and physicians. Better antifungal drugs and diagnostic methods are needed. Advances in *Aspergillus* genomics are giving us new tools for understanding this extremely diverse genus. Hitherto undiscovered sexual stages have been discovered based on findings from genomics. Molecular biologists are trying to understand the mechanisms by which pathogenicity and sexuality work and to deconstruct the physiological pathways that are central to these processes. Evolutionary biologists are focusing on the forces that drive variation within and among population. Economically important species are being re-tested for new capabilities using new screens developed with the aid of post genomic technologies.

**Chapter 2 Molecular Systematics of *Aspergillus* and its Teleomorphs**

Robert A. Samson and János Varga

Mainly because of its economic importance, the genus *Aspergillus* has one of the better described taxonomies among filamentous fungi. Molecular techniques, especially multilocus sequencing studies have recently enabled mycologists to get closer to clarify the evolutionary relationships of the *Aspergillus* genus and its teleomorphs. In this review, we summarize our current knowledge on the systematics of the *Aspergillus* genus, with emphasis on economically important *Aspergilli*. Based on the phylogenetic analysis of multilocus (calmodulin, RNA polymerase 2 and rRNA) sequence data, *Aspergillus* can be subdivided into eight subgenera. Subgenus *Aspergillus* with the sections *Aspergillus* and *Restricti*; subgenus *Fumigati* with the sections *Fumigati*, *Clavati* and *Cervini*; subgenus *Circumdati* with the sections *Circumdati*, *Nigri*, *Flavi* and *Cremeri*; subgenus *Candidi* with section *Candidi*; subgenus *Terrei* with sections *Terrei* and *Flavipedes*; subgenus *Nidulantes* with sections *Nidulantes*, *Usti* and *Sparsi*; subgenus *Warcupi* with sections *Warcupi* and *Zonati* and subgenus *Ornati* with section *Ornati*. At present 13 teleomorph genera are known with an *Aspergillus* anamorph: *Chaetosartorya*, *Dichotomomyces*, *Emericella*, *Eurotium*, *Fennellia*, *Neocarpenteles*, *Neopetromyces*, *Neosartorya*, *Penicilliopsis*, *Petromyces*, *Sclerocleista* and *Warcupiella*. These genera are taxonomically specifically assigned to the various subgenera and sections.

**Chapter 3 Comparative Genomics of *Aspergilli***

Natalie D. Fedorova and William C. Nierman

The recent availability of *Aspergillus* genome sequences (*A. nidulans*, *A. fumigatus*, *A. oryzae*, *A. clavatus*, *A. clavatus*, *A. flavus*, *A. niger*, and *A. terreus*) make this genus a treasure trove for comparative genomic studies. The genomic approaches have provided insights into genome structure and evolution, gene flow in natural populations, and the nature of fungal pathogenicity

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and led to the discovery of potentially beneficial enzymes and secondary metabolites. In addition to these exiting findings, several problem areas have emerged. They include the quality of functional and structural genome annotation, the lack of dedicated web-based interfaces that allow easy access to current genome information, and lack of resources necessary to make continuous improvement to genome sequence and annotation data.

#### Chapter 4 Bioinformatics and Systems Biology of *Aspergillus*

Wanwipa Vongsangnak and Jens Nielsen

We present a review of systems biology studies (i.e. genomics, transcriptomics, proteomics, metabolomics and metabolic modeling) and bioinformatics of aspergilli. We summarize the current status of genome sequencing and Expressed Sequence Tag (EST) sequencing projects for different *Aspergillus* species. Nowadays, 15 *Aspergillus* genomes within ten species have been reported, 11 genomes were completely sequenced and publicly available and 4 genomes are ongoing sequencing projects. Beyond the genome, we further describe how this information can be applied to gain system level information. Using systems-wide techniques, we present different *Aspergillus* metabolic models which have been developed so far. Additionally, several transcriptomics studies have been published as well as there are some studies on proteomics and metabolomics of *Aspergillus*. Today bioinformatics and systems biology research is an important toolbox since it allows enhanced understanding of cellular mechanism of aspergilli and relevant fungi.

#### Chapter 5 Transcriptional Regulation in *Aspergillus*

Tetsuo Kobayashi and Masashi Kato

Filamentous fungi utilize a variety of organic carbon sources and inorganic/organic nitrogen sources in natural environments. The recently completed genome sequencing of several *Aspergillus* species reveals that the species in this genus, *A. oryzae* in particular, have numerous metabolic genes, including those for enzymes involved in the degradation of complex polymers such as carbohydrates and proteins. The existence of such an array of metabolic genes confirms the immense historical contribution of *Aspergillus* in fermentation, food, and enzyme industries as a source of metabolites and enzymes. In general, genes involved in the degradation of complex polymers, catabolism, and biosynthesis of metabolites are regulated by the availability of carbon and nitrogen sources. As can be expected from the vast number of metabolic genes, which suggests the genus' ability to utilize a number of complex polymers and monomers in nature, it possesses a large number of transcription factors, especially those with Zn(II)<sub>2</sub>Cys<sub>6</sub> DNA binding motif, a characteristic feature of *Aspergillus*. In this chapter, we first overview the organization of these transcription factors. Next, we describe several mechanisms of transcriptional regulation with special emphasis on the regulation of plant

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polysaccharide-degrading enzymes and the newly discovered physiological role of the Hap complex. Finally, we list the known *Aspergillus* transcription factors with brief descriptions.

**Chapter 6 Genetics and Genomics of Sexual Development of *Aspergillus nidulans***

**Kap-Hoon Han and Dong-Min Han**

About half of *Aspergilli* species that belongs to ascomycetes are known to have sexuality and most of them are homothallic. A number of cleistothecia are formed in a thallus grown from a single haploid conidia or ascospores. Asci develop within a cleistothecium and eight ascospores are produced in an ascus as a result of meiosis followed by an additional mitosis. Genome-sequencing project revealed that two mating genes (MAT) encoding the regulatory proteins that are necessary for controlling partner recognition in out-cross species of filamentous fungi were conserved in most *Aspergilli* species. The MAT gene products in some self-fertile species were not required for recognition of mating partner at pheromone-signaling stage but required at later stages of sexual development. Various environmental factors such as nutritional status, culture conditions and several stresses, influence the decision or progression of sexual reproduction of self-fertile *Aspergilli*. A large number of genes are expected to be involved in sexual development of *A. nidulans* which can be grouped into several categories according to the development stages, such as the decision of sexual reproductive cycle, mating process, growth of fruiting body, karyogamy followed by meiosis and sporulation process. Complicated regulatory networks including signal transduction pathways and gene expression control may work in each stage and stage to stage linkages. In this chapter, we briefly reviewed the components joining in the regulatory pathways of sexual development, although they comprise only a small part of the whole regulatory networks. Most of the genes characterized act in the reproduction decision stage or early sexual development. Some of them control sexual development positively and some negatively. Several components in signal transduction pathways and protein kinases play some roles in decision of sexual development or in formation of fruiting bodies, although the information on the signal they response to and on the transcription factors they connect with is very poor. Regarding the difficulties for studying sexual differentiation, recent progresses in genomics of *Aspergilli* enlarge the boundaries of genetic and molecular biological understanding of sexual development even in the non-fertile species.

**Chapter 7 Genomics and Secondary Metabolism in *Aspergillus***

**Geoffrey Turner**

The availability of genome sequences from several species of *Aspergillus* has facilitated the identification of a large number of putative secondary metabolism genes and gene clusters which were previously unknown. While some limited prediction of function has been possible through bioinformatics, functional analysis has been necessary to confirm such predictions. Some of the

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putative secondary metabolism genes are not expressed at a sufficient level to detect products. This difficulty can sometimes be overcome by manipulating regulatory and structural genes to obtain expression, or by studying different strains of the same species, since expression can be strain dependent. Comparison of the genomes of *Aspergillus* species has revealed a surprising degree of secondary metabolic diversity within the genus, and provided some insights into how new clusters might evolve. There are also examples of clusters in some *Aspergillus* species for which orthologues are found to be present in other genera, but are absent from other *Aspergillus* species. Any future model for evolution of secondary metabolism must account for this unusual distribution pattern.

Chapter 8 Ecology, Development and Gene Regulation in *Aspergillus flavus*

Gary A. Payne and Jiujiang Yu

*Aspergillus flavus* is one of the most widely known species of *Aspergillus*. It was described as a species in 1809 and first reported as a plant pathogen in 1920. More recently, *A. flavus* has emerged as an important opportunistic pathogen and is now recognized as the second leading cause of aspergillosis in humans. *A. flavus* is known best for its production of aflatoxin, one of the most potent naturally occurring compounds. This discovery launched the modern era of mycotoxicology, and led to aflatoxin being one of the best characterized fungal secondary metabolites. Studies in the major aflatoxin producing species, *A. flavus* and *A. parasiticus*, and in *A. nidulans*, which produces the aflatoxin pathway intermediate sterigmatocystin, have revealed an elegant biosynthetic pathway and a complex network of regulatory controls. In this chapter we review the ecology, pathogenicity, and economic importance of *A. flavus*, and regulatory mechanisms that control its development and mycotoxin formation.

Chapter 9 Functional Systems Biology of *Aspergillus*

Christian Rank, Thomas O. Larsen and Jens C. Frisvad

The genus *Aspergillus* is known for an impressive array of outwards directed functional metabolites or extrolites. The most important of these are accumulated acids, secondary metabolites and extracellular enzymes, widely used in biotechnology and important for mammalian health. *Aspergillus* species are also used as transformation hosts and for bioremediation and bioconversions. Following the full genome sequencing of several species of *Aspergillus*, the fields of comparative genomics, transcriptomics, proteomics and metabolomics are now open for exploration. Annotation of the functions of the different genes and proteins and their interactions is, however, not always simple and we suggest that the fields of exometabolomics and exointeractomics should be more systematically explored.

Chapter 10 Novel Industrial Applications of *Aspergillus oryzae* Genomics

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Keietsu Abe, Kentaro Furukawa, Tomonori Fujioka, Daisuke Hagiwara, Hiroshi Maeda, Jun-ichiro Marui, Osamu Mizutani, Toru Takahashi, Akira Yoshimi, Youhei Yamagata, Katsuya Gomi, and Fumihiko Hasegawa

The *Aspergillus* fungi include the industrial fungi *A. oryzae* and *A. niger*, a human pathogen *A. fumigatus*, an aflatoxin producer *A. flavus*, and the model fungus *A. nidulans*. Because these fungi are important from industrial, medical, and agricultural standpoints, their genome sequences have been determined. After or during the course of their genome sequencing projects, genomic tools such as DNA microarrays for each species and efficient gene targeting methods have been developed. The genomic tools are now available to support functional genomic analysis of each fungus and for discovering industrially, medically, and scientifically important genes in combination with bioinformatics and genomic information. This review describes three examples of novel industrial approaches to using *A. oryzae* genome information and DNA microarrays. (i) *A. oryzae* DNA microarrays have been used to monitor solid-state fermentation, and transcriptome analyses of the process have revealed how *A. oryzae* produces large amounts and a large variety of hydrolytic enzymes. (ii) *A. oryzae* DNA microarrays were used as discovery tools to screen novel proteins that can promote the degradation of biodegradable plastics in combination with hydrolytic enzymes. As a result of this screening, a hydrophobin protein (RolA) and a novel surfactant protein (HsbA) were found. The two proteins can bind tightly to the solid surface of plastics, and subsequently recruit a polyesterase (cutinase) onto the solid (plastic surface)/liquid interface to promote enzymatic hydrolysis of the plastics. (iii) DNA microarrays for *Aspergillus* spp. have been used in functional genomics studies of signal transduction pathways. Some pathway-specific target genes have been assigned by means of transcriptome analyses, and pathway-specific reporters have been constructed from the promoters of the target genes for use in screening antifungal drugs.