Ind J Clin Biochem (Apr-June 2011) 26(2):104–119
DOI 10.1007/s12291-011-0131-5

REVIEW ARTICLE

Allergens/Antigens, Toxins and Polyketides of Important *Aspergillus* Species

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Received: 15 April 2011 / Accepted: 15 April 2011 / Published online: 19 May 2011

Abstract The medical, agricultural and biotechnological importance of the primitive eukaryotic microorganisms, the Fungi was recognized way back in 1920. Among various groups of fungi, the *Aspergillus* species are studied in great detail using advances in genomics and proteomics to unravel biological and molecular mechanisms in these fungi. *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus, Aspergillus nidulans* and *Aspergillus terreus* are some of the important species relevant to human, agricultural and biotechnological applications. The potential of *Aspergillus* species to produce highly diversified complex biomolecules such as multifunctional proteins (allergens, antigens, enzymes) and polyketides is fascinating and demands greater insight into the understanding of these fungal species for application to human health. Recently a regulator gene for secondary metabolites, LaeA has been identified. Gene mining based on LaeA has facilitated new metabolites with antimicrobial activity such as emericellamides and antitumor activity such as terrequinone A from *A. nidulans*. Immunoproteomic approach was reported for identification of few novel allergens for *A. fumigatus*. In this context, the review is focused on recent developments in allergens, antigens, structural and functional diversity of the polyketide synthases that produce polyketides of pharmaceutical and biological importance. Possible antifungal drug targets for development of effective antifungal drugs and new strategies for development of molecular diagnostics are considered.

Keywords Aspergillus species · Allergens · Polyketides

Introduction

Aspergilli are ubiquitous in nature and universal in distribution. The diverse *Aspergilli* group comprises human, animal and plant pathogens, apart from fungi with a plethora of industrial applications. *Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus niger* are known to cause allergic reactions and allergic bronchopulmonary aspergillosis (ABPA) in immuno competent individuals. *A. fumigatus* represents a major cause of morbidity and mortality in the patients of Allergic bronchopulmonary aspergillosis (ABPA) [1]. *A. fumigatus, A. flavus* and *A. niger* are also opportunistic human pathogens in immunocompromised patients such as post transplant cases, HIV etc. where the disease often leads to fatality [2]. A number of novel allergens and antigens of diagnostic and therapeutic importance, multifunctional proteins and toxins have been identified and characterized from *Aspergillus* species, particularly from *A. fumigatus*.

The aflatoxin producing *A. flavus* and ochratoxin producing *A. ochraceus* are plant pathogens infamous for their ability to affect a wide variety of crops. *A. niger* and

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A. oryzae are widely utilized in food industry for citric acid production by fermentation technologies [3, 4]. Lovastatin, the commonly used anticholesterol drug is produced by *A. terreus* while *A. nidulans* is being used as a model organism to study cellular physiology and genetics [5].

Currently, only 30% of the genes of *Aspergillus* species have been characterized leaving the scope for identification of highly diversified biomolecules of human interest [6]. Recent advances in fungal genomics revealed scores of hitherto unknown information of these *Aspergillus* species. This has opened up new avenues to study biological and molecular mechanisms in host pathogen interactions and also for exploration of unknown secondary metabolites of pharmaceutical and commercial importance. One of the important aspects of *Aspergillus* biology is the production of secondary metabolites such as polyketides. Very little is known about the structural, molecular and functional aspects of *Aspergillus* polyketides, their biosynthetic pathways, and the important enzymes involved in these pathways. Polyketide synthase, the key enzyme in polyketide biosynthetic pathways, is a multidomain and multifunctional enzyme.

Recent advances in the understanding of the biomolecules of *Aspergillus* species and the structural and functional diversity of the polyketide synthases useful for applications are reviewed in the article.

Unique Characteristics of the *Aspergillus* Species

Aspergillus mycelium forms conidiophores producing large numbers of conidia (asexual spores) which are dispersed through the air and inhaled by humans. These small airborne spores (2–3 μm in diameter) can bypass mucociliary clearance by human host and cause the disease. *Aspergillus* species can be distinguished from each other by the conidial pigmentation. The conidia of *A. fumigatus* are bluish to green in color; of *A. flavus* and *A. niger* are green and black respectively. The ability to thrive at very high temperature ranges, from 37 to 55°C in soil, mammalian and avian tissues is a unique property of *A. fumigatus* among *Aspergillus* species [7]. This may be due to its metabolic adaptation to higher temperature and presence of higher number of heat shock responsive genes compared to other *Aspergillus* species [7, 8]. To achieve these adaptations, this fungus has evolved distinct mechanisms of stress resistance that may provide basis for its virulence. Apart from its thermotolerant growth, *A. fumigatus* counters hostile environment while retrieving essential nutrients from the environment whether it is a human host or the decaying organic or plant debris, and thus, adapt well to a broad range of environmental conditions. *A. fumigatus* secretes number of catabolic enzymes such as peptidases and proteases, to degrade macromolecular polymers for nutrient uptake from the host [9, 10].

Interesting Biomolecules of *Aspergilli*

Aspergillus species are a continuous source of complex proteins, allergens, antigens and enzymes to the environment. *A. fumigatus, A. flavus* and *A. niger* are known to cause Type I and Type III hypersensitivity reactions in humans. *A. fumigatus* is known to produce multifunctional enzymes and toxins that facilitate the adherence and hydrolysis of the components of the host cell, and complex allergens which cause severe allergic reactions. In view of the clinical importance of the allergens/antigens of *Aspergillus* species in the allergic and invasive disease, over 20 genes encoding *A. fumigatus* antigens have been recombinantly expressed and evaluated for their diagnostic importance (Table 1) [11–17]. So far, 34 allergens of *A. fumigatus,* from *Asp f1* to *Asp f34,* have been designated by IUIS Allergen Nomenclature Committee [18]. Asp f 35 (34 kDa protein with unknown function), Asp f 36 (extracellular arabinase), and Asp f 37 (chitosanase) from *A. fumigatus* are recently accepted to be included in the list [19]. Asp fl, an 18 kDa allergen/antigen, is a major cytotoxin secreted by *A. fumigatus.* Multiple roles of this 18 kDa protein such as allergenicity, antigenicity, ribonuclease activity and cytotoxicity have been iterated and this allergen is implicated in pathogenesis [20, 21]. Few more allergens such as 44-kDa allergen, *Aspf23* (L3 ribosomal protein), 40 kDa protein disulphide isomerase, 45 and 56kDa glycoprotein antigens and a 34 kDa allergen with protease activity were characterized and were implicated in virulence [22–24]. Among *Aspergillus flavus* allergens Asp fl 13, an alkaline serine protease and Asp fl 18, vacuolar serine proteases were characterized. These proteases and protease isozymes have been implicated in colonization of animal host. Genes with >90% sequence homology with Asp f 1, Asp f 5, Asp f 12, Asp f 22 and Asp f 23 were mapped on *A. flavus* genome [25]. However, these allergens are not yet reported from *A. flavus* and need to be characterized. Alkaline phosphatases of *A. flavus* are implicated in aggressive colonization of cotton balls, and hydrolases of the same are considered as pathogenic factors for plants [26, 27].

Advances in the field of genomics and proteomics contributed immensely to better understanding of the host-pathogen factors and their interactions in *Aspergillus* and other fungal diseases. Some of the promising advances include molecular interaction studies of lung surfactant proteins SP-A, SP-D and Mannan binding lectin (MBL) with *A. fumigatus.* These studies are based on in vitro cell culture experiments and murine models

Table 1 *Aspergillus fumigatus* allergens/antigens of diagnostic relevance

Allergen	Biological activity	Use of recombinant allergen for diagnosis
Asp f 1[a] (18 kDa)	Allergic, ribonuclease activity, cytotoxicity	IgE reactivity
		IgG reactivity
		Immunodominant peptides
Asp f2[a] (37 kDa)	A fibrinogen-binding protein	IgE reactivity
		Immunodominant peptides
Asp f3[a] (19 kDa)	Peroxisomal membrane proteins (PMP)	IgE reactivity
Asp f4[a] (30 kDa)	Unknown biological function with	IgE reactivity
		Immunodominant peptides
Asp f5 (42 kDa)	Metalloprotease (MEP)	IgE reactivity
Asp f 6[a] (23kD)	Manganese superoxide dismutase (MnSOD)	IgE reactivity
		Immunodominant peptides
Asp f 8[a] (11.1 kDa)	A glycoprotein with homology to ribosomal protein P2	IgE reactivity
Asp f 9 (18.8 kDa)	Glycosyl hydrolase	IgE reactivity
Asp f 10 (34.4 kDa)	Aspartic proteases (PEP)	IgE reactivity
Asp f 11 (95 kDa)	Cyclophilin or a dipeptidyl-peptidase IV	IgE reactivity
Asp f 16 (43 kDa)	A protein with unknown function and similarity to Asp f 9.	IgE reactivity
		Immunodominant peptides
Asp f 23 (44 kDa)	Ribosomal protein L3	IgG reactivity

[a] *Aspergillus fumigatus* allergens are used in ImmunoCap method for serodiagnosis or skin testing

including knockout mice of ABPA and invasive aspergillosis [28–31]. Therapeutic potential of recombinant human surfactant protein D (rhSP-D) has been established by studies in murine models of lung allergy, hypereosinophilic SP-D gene-deficient mice and also with eosinophils from allergic patients. These studies clearly suggest therapeutic application of rhSP-D for allergic and invasive diseases [32–37]. Single nucleotide polymorphisms (SNPs); have been observed in these genes (SP-A, SP-D and MBL). Such polymorphisms may result in partial or total loss of function and may contribute to the hosts' susceptibility to aspergillosis. Studies showed association of SNPs in SP-A2 and MBL genes with patients of allergic bronchopulmonary aspergillosis and bronchial asthma with rhinitis [38]. Adhesin proteins of *A. fumigatus* have been recently identified and characterized. Adhesin protein of *A. fumigatus*, Extracellular thaumatin domain protein (AfCalA) was recombinantly expressed and specific binding with laminin and murine lung cells was established [39]. This suggests that the mechanism of binding of *Afu* conidia to the host cells is through adhesin proteins of *A. fumigatus*. Host-pathogen interaction studies of *A. flavus* have been carried out with the plant cells using microarray and proteomics technology. However, host–pathogen interactions with respect to *A. flavus* and *A. niger* and animal/human host need to be studied in detail.

Virulence of *A. fumigatus* is not attributed to a single protein/gene and appears to be a multifactorial trait. Cell wall molecules like β-glucan, α-glucan, chitin, galactomannans, galactomannan proteins (Afmp1p, Afmp2p), hydrophobins (rodA/hyp1 and rodB) and DHN-melanin of *A. fumigatus* are known to interact with the host and alter the immune responses. The genes for β (1-3) glucan synthases (Fks1p), glucanosyltransferase (Gel 1p, Gelp 2p, and Gel3p), β (1-3) endoglucanase (Engl1), α (1-3) glucan synthetase, and chitin synthase (ChsE, ChsG) are studied for their contribution in virulence by gene complementation studies [40]. Genes and proteins/enzymes reported to have a role in the resistance to innate immune response belong to conidial specific catalases (catA, cat1/catB, cat2/katG, catC, and catE), superoxide dismutases (sod1, sod2, sod3/asp f 6, and sod4), fattyacid oxygenases (ppoA–C), glutathione tranferases (gstA–E, afyap1, skn7, and pes1), efflux transporters (mdr1–4, atrF, abcA–E, and msfA–E) and DHN-melanin cluster genes (*pksP/alb1, arp1, arp2, abr1, abr2*, and *ayg1*) [41]. Melanin, a pigment (1,8-dihydroxynaphthalene) of *A. fumigatus* is considered as a virulent factor as it protects the integrity of the conidial cell wall. This also helps in the expression of adhesins at the conidial surface necessary for binding to host cells [42]. Cyclic AMP-dependent Protein Kinase A (PKA) signal cascade is also found to be a critical regulator for conidiation, development, growth, and stress responses [43]. *A. fumigatus* toxin associated with conidia is fumigaclavine

C, an alkaloid metabolite and potent inhibitor of DNA synthesis. It also secretes a number of low molecular weight toxins such as gliotoxins, helvolic acid, verruclogen, a protein toxin Asp f1 that contribute to the virulence and also toxins such as fumagillin, and fumitremorgin A–C (Neurotropic toxins).

Drug Target Pathways/Genes

A. fumigatus genome sequencing coupled with the genetic tools for gene manipulation and high-throughput microarray analysis has facilitated the research for novel antifungal drug targets. The drugs currently available for invasive aspergillosis (polyenes, azoles, and echinocandins) target synthesis of cell wall molecules (ergosterol, β-1,3 glucan, and chitin) or molecules involved in the steps for synthesis. This approach has limitations due to the structural homology with cholesterol in the human host and results in toxicity and development of drug resistance by the pathogen (Fig. 1). Unique biochemical pathways and their key enzymes in *Aspergillus* species which are absent in humans are being explored by various experimental strategies for identification of novel drug targets. The glyoxylate bypass, methylcitrate cycle, lysine biosynthetic pathway and trehalose biosynthetic pathway are few examples (Table 2). New strategies using advanced genomic tools such as conditional promoter replacement (CPR), gene replacement and conditional expression (GRACE) strategies lead to identification of number of essential genes for fungal growth. These can be explored for development of antifungal drug targets [44]. Polysaccharide components of fungal cell wall and its biosynthetic pathway have been attractive targets for selective antifungal drug development [45, 46]. In contrast to yeast cell wall *A. fumigatus* lacks β-1,6 glucan, which interconnects proteins, chitin, and β1-3 glucans and also lacks cell wall bound homologous GPI-proteins [Glycosylphosphatidylinositol (GPI)-anchored proteins (GPI-Aps)] linked to glucans, important for cell wall organization. Instead, *Afu* contains hydrophobins that are important for conidial survival and attachment to hydrophobic surfaces [47] that can serve as potential drug targets in *A. fumigatus*. The methylcitrate cycle, is essential for degradation of toxic propionyl-CoA via methylcitrate synthase enzyme in fungi and some bacteria. *A. fumigatus* methylcitrate synthase gene transcription was reported during invasion from infected mouse lungs. Virulence of *A. fumigatus* was attenuated by deletion of methyl citrate synthase gene. Therefore, methylcitrate cycle which is absent in humans may provide a potential drug target [48, 49]. Glyoxylate pathway was implicated in the pathogenicity of other pathogenic organisms such as *Candida albicans* and *Mycobacterium tuberculosis* [50]. Mutants of *A. fumigatus* that do not have isocitrate lyase of glyoxylate pathway retained the same virulence as the wild-type in *A. fumigatus* [51]. This confirms that glyoxylate cycle is not important in the anaplerotic synthesis of oxaloacetate in *A. fumigatus* under infectious conditions [52]. Trehalose is a non-reducing disaccharide and functions as a reserve carbohydrate and a stress metabolite which also protects the fungal cell by preventing aggregation of denatured proteins and scavenging free radicals [53, 54]. Trehalose biosynthesis has been linked to virulence in pathogenic fungi and is absent in mammalian cells. Thus, it could be a potential target for antifungal therapy. Trehalose content increases during the life cycle of *A. fumigatus* and also after heat shock but not in response to other types of stress.

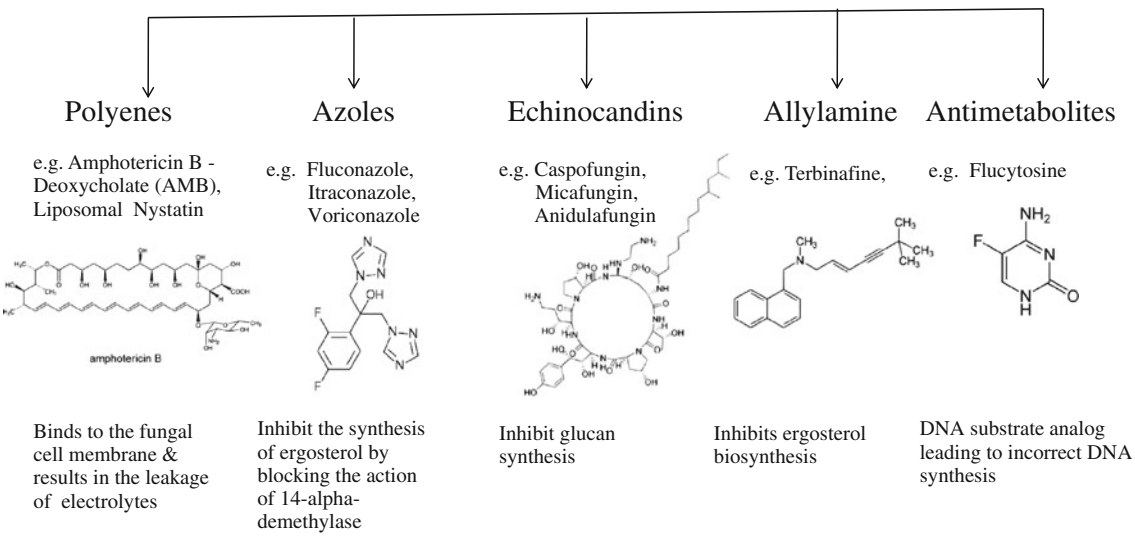


Fig. 1 Antifungal drugs and the mechanism of action

Table 2 Biochemical pathways of *Aspergillus* species targeted for therapy

Name of the fungus	Biochemical pathway	Important role	+/− in humans	Current drugs	Limitations
A. fumigatus, C. neoformans	Ergosterol biosynthesis	Integral part of cell membranes	−ve	Amphotericin B, azoles, Echinocandins	Acts also on cholesterols, due to structural similarity
A. fumigatus	Methyl citrate cycle	For degradation of toxic propionyl-CoA	−ve		–
A. fumigatus, C. albicans, S. cerevisiae, C. neoformans	Glyoxylate pathway	Serve as carbon sources in gluconeogenesis from fat	−ve		Not essential for survival
A. fumigatus, C. neoformans	Lysine biosynthesis	Essential amino acid	−ve		Lysine biosynthesis may not be required for invasive growth
A. fumigatus, S. cerevisiae	Trehalose biosynthesis	Reserve carbohydrate source	−ve		Virulence mechanism not known clearly
A. fumigatus, C. neoformans	Melanin biosynthesis	Conidial pigment and virulent factor	−ve		Not essential for survival

Using gene disruption studies, tpsA and tpsB genes of *A. fumigatus* were found to be involved in the trehalose biosynthesis [55]. Deletion of the two genes led to delayed germination of conidia at 37°C and conidia were susceptible to oxidative stress. Although, trehalose synthesis is related with pathogenicity in *Afu*, the mechanism of virulence is not clearly understood [56]. Another pathway, alpha-aminoadipate pathway, essential for lysine biosynthesis which is absent in human, has also been suggested as a potential antifungal drug target for *A. fumigatus*. Deletion mutant of lysine pathway-specific enzyme, homocitrate synthase (HcsA), was found to be attenuating virulence in a corticosteroid-based murine infection model of bronchopulmonary aspergillosis. However, the supply of excess lysine via the drinking water partially restored virulence, implying importance of lysine auxotrophy in virulence [57]. Comparative genomic analysis, manual mining of experimentally confirmed essential genes from fungal pathogen (such as *C. albicans* or *A. fumigatus*) and modeling studies proved helpful in drug target discoveries. Four such genes were recently identified as putative drug targets in *A. fumigatus* with other fungal pathogens such as *Candida albicans*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Paracoccidioides lutzii*, *Coccidioides immitis*, *Cryptococcus neoformans* and *Histoplasma capsulatum* which were absent in the human genome. These genes are: *trr1*, that encodes for thioredoxin reductase, rim8, encoding a protein involved in the proteolytic activation of a transcriptional factor, kre2, that encodes for α-1,2-mannosyltransferase and erg6, that encodes for D-(24)-Sterol C-methyltransferase [58]. The validity of these genes as drug targets needs to be experimentally established for development of novel antifungal drugs.

Genes and Genomics of *Aspergilli*

Important Secondary Metabolites: Polyketides

Genome sequencing has facilitated a great insight into the secondary metabolite genes for a comprehensive study. Genome size of important *Aspergillus* species varies from 30–40 Mega bases (Mb) spanned on eight chromosomes with good synteny among species (50%). *Aspergillus* species present unique genes which vary from 140 to 500 in numbers for different *Aspergilli* (Table 3). Currently, only <10% of the 9,000–13,000 open reading frames (ORFs) have been assigned with functions [59–61]. However, a large number of genes are unknown with respect to function. Till date most of the transcriptome of *Aspergillus* has been predicted based on the bioinformatic approach and needs to be validated in vivo. *A. fumigatus* (*Af293*) genome sequencing revealed nine previously unknown allergens, identification of numerous genes involved in the production of specific secondary metabolites, and a set of essential genes that may be potential targets for drug development [62]. Genome-sequencing projects of *Aspergillus* species and the web-based bioinformatics tool SMURF (Secondary Metabolite Unknown Regions Finder; www.jcvi.org/smurf) have shown that each species of *Aspergillus* genome contain 30–70 secondary metabolite clusters which includes 15–35 polyketide synthases, 12–30 non ribosomal peptide synthases, and a number of dimethylallyl tryptophan synthases (DMATS) [63, 64] (Table 3).

Aspergillus fumigatus shows presence of 28–30 clusters of secondary metabolites. Melanin gene cluster has been identified and characterized from *Afu*. Several other secondary metabolite gene clusters are characterized in

Table 3 Genes and genomics of *Aspergillus* species

	A. fumigatus	*A. nidulans*	*A. oryzae*	*A. niger*	*A. flavus*
Strain	Af293	FGSC A4	ATCC 42149	CBS 513.88	NRL 3357
Genome size (Mb)	28.81	30.06	36.7	33.97	36.51
Number of chromosomes	8	8	8	8	8
Number of protein coding genes	9630	9541	14063	14097	13515
Annotated proteins (Pfam hits)	5808	4512	10416	5306	5510
Unique genes	148	469	331	236	278
Probable PKS genes	21	24	27	34	25
Secondary metabolite clusters	28–30	46	56	67	55

A. fumigatus which produce gliotoxins (immunosuppressive and proapoptotic for mammalian cells) [65], fumigaclavines (ergot alkaloid used for therapeutic use) [66], fumitremorgins (mycotoxin) [67], and siderophores [68]. Gliotoxin, a secondary metabolite produced via gliotoxin biosynthetic pathway using non-ribosomal peptide synthase machinery, has been found in lungs of invasive aspergillosis patients with cancer and also in IA mice model [69]. Other mycotoxins involved in mycosis caused by *A. fumigatus* are trypacidin, verruculogen and fumigaclavine A. Further metabolomic studies on *A. fumigatus* suggest that it produces number of extrolites of different chemical nature including polyketides, non ribosomal peptides, terpenes, anthroquinones and compounds of mixed origin with aminoacids, such as siderochromes etc. [70]. Annotation of the *A. flavus* genome indicates that it has 55 secondary metabolite biosynthetic pathways which may cross link with other pathways to generate various metabolites. So far, only Aflatoxin and cyclopiazonic acid gene clusters have been characterized from *A. flavus*. Genome sequencing of industrially important species, *A. niger* revealed presence of highest number of secondary metabolite clusters that is 67, with 34 polyketide synthases and 17 non-ribosomal peptide synthases arranged in clusters [71]. For *A. niger* a link between genes involved in secondary metabolite synthesis has only been determined for siderophore and spore pigmentation so far [72]. *A. niger* genome sequencing also showed homology with gene sequences of fumonisin pathway from *Gibberella moniliformis* indicating its potential to produce mycotoxin such as fumonisin [73]. However, *A. niger* does not produce this toxin. Some *A. niger* strains are known to produce ochratoxin but very little is known about the biosynthetic pathway of this mycotoxin in this species. *A. oryzae* useful for food industry has potential for a remarkably large number of secondary metabolites compared to pathogenic fungus *A. fumigatus* and *A. flavus*. It has high number of secondary metabolite genes which are enriched in regions lacking synteny with either *A. fumigatus* or *A. nidulans* indicating *A. oryzae*'s capability to produce specific metabolites. This is also supported by the presence of 56 secondary metabolite clusters and highest no. of cytochrome P450 genes in the *A. oryzae* genome. Recently, a novel polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) gene involved in cyclopiazonic acid (CPA) production has been identified from *A. oryzae* [74]. The presence of putative genes encoding other principal enzymes involved in phenylpropanoid and flavonoid biosynthesis (such as phenylalanine ammonia-lyase, cinnamic acid hydroxylase and p-coumarate CoA ligase) in *A. oryzae* genome prove the presence of fungal phenylpropanoid-flavonoid metabolite pathway in industrially useful *A. oryzae* [75]. Although, *A. oryzae* is not known to produce aflatoxin, aflatoxin biosynthetic clusters genes have been mapped on its genome and these genes appear to be cryptic or silent. Genome sequence of several isolates of *A. oryzae* when compared with *A. flavus*, showed presence of aflatoxin biosynthesis genes with deletions, frame shift mutations, and base pair substitutions [76]. These alternations seem to be responsible for the inactive aflatoxin genes in *A. oryzae*.

Secondary metabolite pathway genes in *Aspergillus* species are conveniently arranged in gene clusters with transcriptional regulators, mostly at the end of chromosomes, and have been predicted to be involved in the synthesis of various polyketides or non-ribosomal peptides. However, most of these clusters are silent or cryptic, under the laboratory conditions with the exception of few toxins and a virulent factor. To activate expression of silent clusters and to identify the metabolites produced, different strategies based on molecular, epigenetics and cultivation methods are undertaken in *Aspergillus* field [77, 78]. The novel global regulator of secondary metabolite, LaeA, is recently described in *Aspergilli* by Keller et al. Loss of this gene eliminates sterigmatocystin, aflatoxin, lovastatin, and gliotoxin production in *Aspergillus* species and results in an avirulent *A. fumigatus* mutant strain [79, 80]. LaeA may affect, histone methylation, and this might alter the state of chromatin in metabolite gene clusters. However, the role of this protein in regulation of secondary metabolites has not yet been established fully [81]. In view of the importance

of various polyketides, the structural and functional characterization of genes and proteins of the polyketide synthases (PKS), a key enzyme in polyketide biosynthetic pathway in the *Aspergillus* species needs to be studied in detail.

Aspergillus Polyketides

Polyketides are a large and diverse group of natural products including polyphenols, polyenes, and macrolides with a wide variety of biological activities with antibiotic, antifungal, and anticancer properties. They are chemically distinct group containing multiple –CH2–CO– ketide group synthesized by repetitive condensation or polymerization reactions [82]. *Aspergillus* species produce a number of secondary metabolites of varied biochemical structures such as terpenes, alkaloids, ergots and polyketides. *Aspergillus* polyketides are basically naphtho-γ-pyrone and furanocoumarin ring compounds. Important polyketides produced by *Aspergillus* species are classified as (i) drugs and polyketides of pharmaceutical relevance and (ii) virulent factors and toxins (Table 4). Pharmaceutically relevant polyketides includes cholesterol lowering lovastatins by *A. terreus*. *A. nidulans* is also known to produce number of polyketides with antimicrobial and anticancer property [83, 84]. Polyketides as virulent factors and toxins comprise melanin pigments from *A. fumigatus*, carcinogenic mycotoxins aflatoxins from *A. flavus*, and *A. parasiticus* and sterigmatocystin from *A. nidulans*. Aflatoxin and sterigmatocystin are hepatotoxic, neurotoxic and cause threat to the human health. Mycotoxin contamination of the agricultural crops and agri products leads to great economic losses, as the permeable limit of Aflatoxin (4–20 ppb) for food and food products in international trade has been fixed in view of the WTO stipulations [85]. Important mycotoxins of agricultural crops produced by *Aspergillus* species include Aflatoxins, ochratoxin and cyclopiazonic acid.

Polyketide Synthases in *Aspergillus* Species

Polyketide synthases are present in fungi and bacteria. They are the key enzymes in the polyketide biosynthetic pathway. Polyketide synthases are classified in three types based on their structural architect of the domains and their functional use (Table 5). Type I PKS enzymes are large multifunctional proteins of <1000 AA and are encoded by a single gene in fungi and bacteria e.g. PksA of *A. flavus*. Type II PKS enzymes are dispersed as individual proteins of approximately 500 AA and are found in bacteria e.g. actinorhodin PKS of *Streptomyces coelicolor*. It is an antibiotic substance from *Streptomyces coelicolor* [86]. Type III polyketide synthases are comparatively small proteins of 350–500 AA with a single polypeptide chain and are involved in the biosynthesis of precursors for flavonoids like chalcones and stilbenes in plants [87].

Aspergillus PKS enzymes are Type I and consist of five to nine domains on single polypeptide chain of 1000–3000 AA (Fig. 2). These PKS enzymes use an iterative strategy i.e. each domain is used repeatedly to extend the polyketide chain to build polyketide e.g. Polyketide synthase A (Pks*A*) for Aflatoxin and Polyketide synthase *P* (PksP) for melanin production. However, Lovastatin diketide synthase (LovF/LDKS) of *A. terreus* acts non-iteratively like the bacterial modular PKS enzymes [88]. Bacterial PKS enzymes use modular architect, where domains are organized into modules and used for the catalysis of one cycle of polyketide chain elongation non-iteratively as in the case of 6-deoxyerythromycin B synthase (DEBS) for the biosynthesis of erythromycin in streptomyces species [89, 90]. Interestingly, apart from Type I PKS enzymes, recently Type III PKS genes resembling the chalcone synthase genes (CHS) for the production of the flavonoid have been identified in *A. flavus* and *A. oryzae* by comparative genome analyses [89, 90].

Domain Organization of *Aspergillus* PKS Enzymes

The diversity in polyketide structure and function in *Aspergilli* is reflected in the structural diversity of PKS enzyme and the Polyketide biosynthetic pathway (Fig. 3). *Aspergillus* PKS enzymes use substrates such as acetyl-CoA and malonyl-CoA/hexonyl CoA in a similar manner to multi-domain Fatty Acid Synthases but differ by incomplete reduction and/or dehydration steps [91]. A range of domains of the PKS enzyme facilitate different steps in the synthesis of various intermediates of polyketide products. The core domains essential for PKS enzymatic machinery are (i) β-keto synthase (KS) domain for decarboxylative condensation between an acyl CoA and a malonyl CoA, (ii) an acyl carrier protein (ACP) domain for carrying malonyl co-A loading by phosphopantetheinyl arm and (iii) an acyltransferase (AT) domain for carboxylic acid unit, selection and transfer onto the KS. The presence of other domains like β-ketoacyl reductase (KR), enoyl reductase(ER), dehydratases (DH), and methyltrasferase (MT) and CYC/TE is variable in different Polyketide synthases in different *Aspergillus* species. During polyketide biosynthesis, the newly formed β-ketothiolester is subjected to a series of chemical reactions like reduction by a β-ketoacyl reductase (KR) to a secondary alcohol; dehydration to form an unsaturated thiolester; methylation,

Table 4 Polyketides and polyketide synthases of *Aspergilli* and diversity in the KS domain*

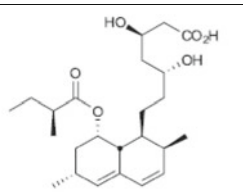
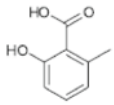
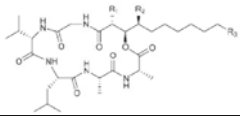
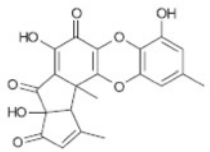
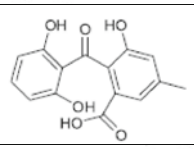
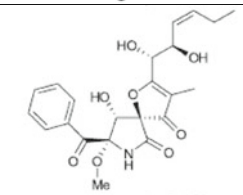
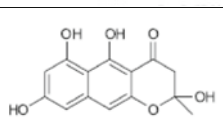
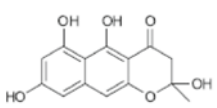
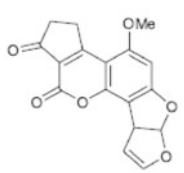
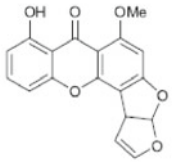
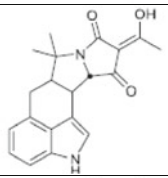
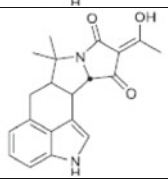
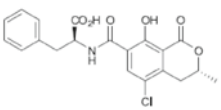
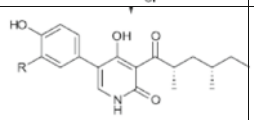
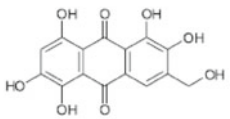
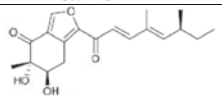
Polyketide and its use	*Aspergilli* species	Chemical structure	Gene and protein Accession no.	Domain Architect	% homology[f]
Lovastatin (anticholeresterol agent)	*A. terreus*		*lovB*[a] (AAD39830.1)	KS-AT-DH-MT-(ER)-KR - ACP-CON	35%
			lovF[a] (AAD34559.1)	KS-AT-DH-MT-(ER)-KR - ACP	34%
6-MSA (precursor to lovastatin)	*A. terreus*		*atX*[b] (XP_001215453.1)	KS-AT-DH-CON-KR-ACP	33%
Emericellamides (antibiotic compound)	*A. nidulans*		*easB*[c] (tpelCBF87072.1l)	KS-AT-DH-MT-(ER)-KR - ACP	33%
Orsellinic acid , F9775A, F9775B (anticancer property)	*A. nidulans*		*orsA*[c] (tpelCBF75781.1l / AN7909.4)	SAT-KS-AT-PT-ACP-ACP-TE	37%
Monodictyphenone (antimicrobial property)	*A. nidulans*		*mdpG*[c] (CBF90097.1, AN0150)	SAT-KS-AT-PT-ACP	55%
Pseurotin A (competitive inhibitor of chitin synthase, and a neuritogenic Agent)	*A. fumigatus*		*psoA*[c] (PKS–NRPS hybrid) (XP_747151.2)	KS-AT-DH-MT-KR- ACP-C-A-PCP-R	35%
Melanin (conidial pigment and virulent factor)	*A. fumigatus*		*pksP*[c] (XP_756095.1)	SAT-KS-AT-PT-ACP-ACP-TE/CLC	64%
YW1 (conidial pigment)	*A. nidulans*		*wA*[c] (prf|l1905375A)	SAT-KS-AT-PT-ACP-ACP-TE/CLC	65%
Aflatoxin (Mycotoxin)	*A. flavus and A. parasiticus*		*pksA*[c], *pksL*[c] (AAS90093.1)	SAT-KS-AT-PT-ACP-TE/CLC	100%

Table 4 continued

Sterigmatocystin (mycotoxin)	*A. nidulans*		*pksST*[c], *stcA*[c] (Q12397.2)	SAT-KS-AT-PT-ACP-ACP-TE/CLC	99%
Cyclopiazonic acid (Mycotoxin)	*A. oryzae*		*cpaA*[c] (PKS–NRPS hybrid) (XP_001818816.2)	KS-AT-(DH)-(MT)-(ER)-(KR)-ACP-ACP-C-A-PCP-DKC	31%
Cyclopiazonic acid (Mycotoxin)	*A. flavus*		*cpaA*[c] (PKS–NRPS hybrid) (BAI43678.1)	KS-AT-(DH)-(CMeT)-(ER)-(KR)-ACP-ACP-C-A-PCP-DKC	35%
Ochratoxin A (Mycotoxin)	*A. ochraceus*		*Pks* (AAP32477.1)	ACP-DH	67%
Aspyridone	*A. nidulans*		*apdA*[d] (tpelCBF80487.1)	KS-AT-DH-CMeT-(ER)-KR-ACP-C-A-PCP-DKC	32%
Asperthecin	*A. nidulans*		*aptA*[c] (XP_663604.1/ AN6000.2)	SAT-KS-AT-PT-ACP	53%
Asperfuranone	*A. nidulans*		*afoE* (tpelCBF88295.1/ XP_658638)	SAT[g]-KS-AT-DH-MT-PT-R	35%

[a] Highly reducing polyketide synthase

[b] Partially reducing PKs enzyme

[c] Non reduced PKS enzyme

[d] Non ribosomal peptide synthase (NRPS) enzyme

[e] Hybrid PKS–NRPS enzyme

[f] % of KS domain homology with PksA protein (AAS90093.1)

[g] % of SAT domain homology in *afo*E[a] is 27%

* Reference 89

using a methyl group from *S*-adenosylmethionine (SAM) and final enoyl reduction (ER) to form a fully saturated thiolester. Polyketide structural diversity is determined by iterative activity of MT (CMeT), DH and ER domains during each round of extension. However, how those multi-functional enzymes control the product chain length and the site of iteration is largely unknown. In addition, post-translational modifications of the basic polyketide skeleton, by tailoring enzymes such as methyltransferases, esterases, glycosidases and dimerization with other polyketides, also contribute to the diversity of the polyketide compounds. Polyketide synthases are classified as non reducing (NR) and reducing Polyketide snyhthases based on absence of reducing domains [partially reducing (PR) and highly reducing (HR) PKS] [92]. The type of compounds they produce also depend on the functionality of reducing domains like MT, DH, ER, and TE/CLC (thioesterase/ Claisen cyclase) present in the enzyme which are classified as Partially reduced or highly reduced PKS producing highly, reduced polyketide compounds such as 6-methyl-salicylic acid and lovastatins. Non reduced PKS enzyme produces compounds without reduction reactions. They

Table 5 Diversity of polyketide synthases in microbes

Microbes	Type of polyketide synthase	Protein structure	Functional characterization	Types of polyketides produced	Domain organization
Bacteria	Type I (modular)	Single linear protein with multiple modules	Active site used only once	Macrolide polyketides, such as the erythromycin A and rifamycin	e.g. Saccharopolyspora erythrea KS-AT-ACP-KR-TE = KS-AT-ACP-KR-TE = KS-AT-ACP-KR-TE = KS-AT-ACP-KR-TE
Bacteria	Type II (Iterative)	Multiple proteins, with a single mono-functional active site	Active sites may be used only once or repeatedly	Aromatic polyketides, such as actinorhodin and tetracenomycin	e.g. *Streptomyces coelicolor* KS-AT-ACP-KR-DH-ER
Bacteria	Type III (Iterative)	One protein with multiple modules	Active sites are reused repeatedly	Dihydroxyphenylalanine melanins	e.g. bacterial PKS *Streptomyces griseus* (RppA) KS-AT-ACP-TE
Fungi	Type I (iterative)	Single protein with one module	Active sites are reused repeatedly	Lovastatins, Aflatoxins, Melanin	e.g. polyketide synthase (LovF) from *A. terreus* KS-AT-DH-MT-(ER)-KR -ACP
Fungi	Type III	One protein with multiple modules	Active sites are reused repeatedly	3,5-dihydroxybenzoic acid	e.g. CsyA from *A. oryzae* KS-AT-ACP-KR-MT-DH-ER
Plants	Type III (Iterative)	One protein with multiple modules	Active sites are reused repeatedly	Flavonoids like chalcones and stilbenes	e.g. Chalcone synthase from *Medicago sativa*

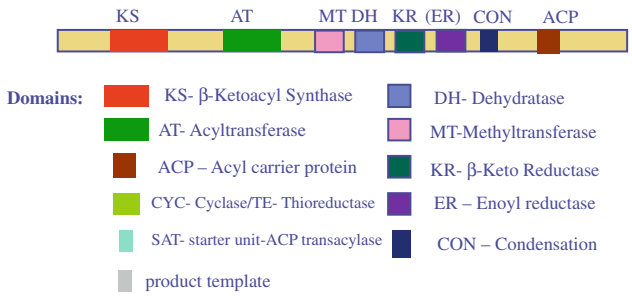


Fig. 2 Lovastatin nonaketide synthase(LovB) of *Aspergilllus terreus* showing general *Aspergillus* Polyketide synthase domain Architect (*Domains like MT, ER,DH,CON are absent in other *Aspergillus* PKS enzymes and may have SAT, PT, and TE/CYC domains)

lack above mentioned reducing domains and instead contain SAT (starter unit-ACP transacylase) and PT (product template) domains which control the structural outcome of non-reduced polyketides compounds such as Aflatoxin and melanin [93]. This diversity in polyketides produced by different *Aspergillus* species suggests the possible occurrence of variations in the functionality and also molecular structure of the important enzymes in the polyketide biosynthetic pathways. This is evident in the synthesis of melanin, aflatoxin, and lovastatin by *A. fumigatus*, *A. flavus* and *A. niger* respectively.

Biochemical Pathways and Enzymes

Melanin—Pigment and Virulent Factor

Polyketide Synthase p (PKS P/*alb1*) of *A. fumigatus* is well characterized and known to synthesize bluish to green pigment, melanin, by using dihydroxynapthalene (DHN)—melanin pathway [94]. Melanin biosynthesis involves 6 genes, organized in a cluster on chromosome 2. These genes are (i) pigment biosynthesis protein (Ayg1), (ii) polyketide synthase (Alb1/PksP), (iii) THN reductase (Arp2), (iv) scytalone dehydratase (ArP1p), (v) vermelone dehydratase (Abr1p), and (vi) laccase (Abr2P). Melanin synthesis in *Afu* starts with condensation of acetyl-CoA and malonyl-CoA, which are converted by the products of the genes PksP/Alb1 and Ayg1 into 1,3,6,8 tetrahydroxynaphthalene (THN) [95]. *pksP*, non reduced polyketide synthase has domains like SAT-KS-AT-PT-ACP-ACP-TE/CLC and it lacks the reducing domains. Conidia lacking pigmentation due to the defective polyketide synthase *pks* P gene were less resistant to the attack by monocytes in vitro and such conidia showed reduced virulence in a murine model of aspergillosis [96, 97]. *pks* P, is transcribed

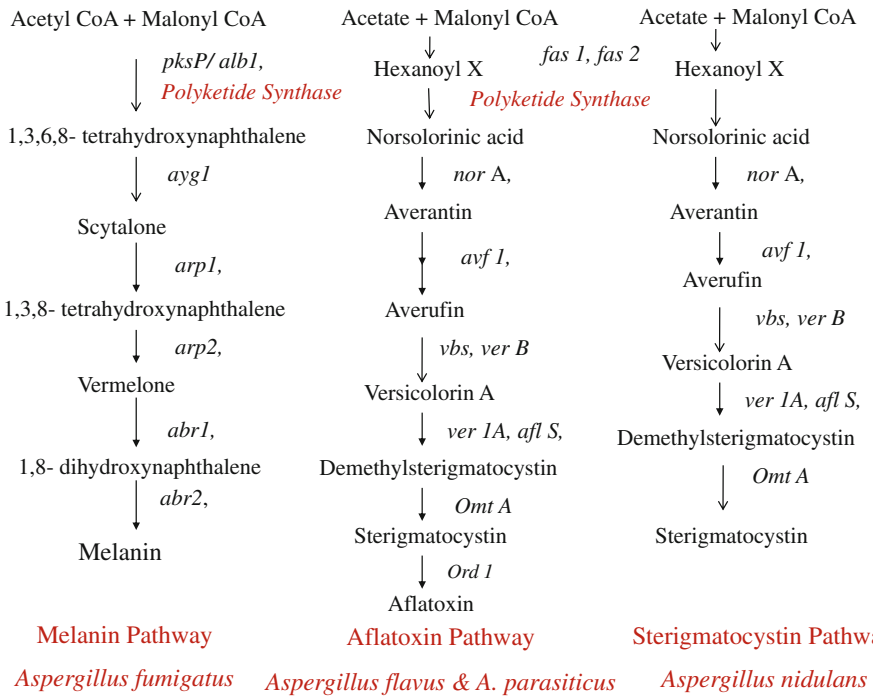


Fig. 3 Polyketide biosynthetic pathway in *Aspergillus* species

in hyphae of germinating conidia isolated from lungs of infected immunosuppressed mice and is also involved in the inhibition of phagosome-lysosome fusion [98]. In addition to a protective role against the host's immune defenses, melanin is also a structural component of the conidial wall that is required for arrangement of correct assembly of the cell wall layers and for expression of adhesins and virulence factors at the conidial surface [96]. Recently, it was also shown that *A. fumigatus* produces an alternative type of melanin, designated as pyomelanin, via tyrosine degradation [99]. *A. niger* produces a black pigment aspergillin; found to be a mixture of a low-molecular weight green pigment hexahydroxyl pentacyclic quinoid (HPQ) and a brown color low-molecular weight melanin pigment [99]. PKS ortholog of alb1 from *A. fumigatus* is responsible for production of the naphtho-γ-pyrone precursor for the 1,8-dihydroxynaphthalene (DHN) melanin/spore pigment in *A. niger* too. In contrast to *A. fumigatus*, the genes for melanin biosynthesis are not clustered in *A. niger*. However, *alb*A and *ayg*A are present on chromosome 1, they are located on different chromosomal arms [100]. *A. nidulans* produces yellow spore pigments, made of naphthopyrones which are reported to be important for scavenging reactive oxygen species for protection of conidia against oxidative damage [101]. In contrast to conidial mutant of *A. fumigatus* where the absence of conidial pigment results in structural changes on the conidial surface, *A. nidulans* mutant strain (wA) does not show the same structural changes on the conidial surface. The biosynthetic pathways for the pigment formation in the conidia of the two species differ, i.e., *A. fumigatus* produces the conidial pigment via the 1,8-dihydroxynaphthalene (DHN)-melanin pathway, which appears to be absent in *A. nidulans* [98]. The pigment synthesis pathway in *A. nidulans* is still not yet established. Genome sequencing of *A. flavus* suggest that it has a homolgous *pks* P/*alb*1 gene and also additional genes required for pigment biosynthesis. However, these remain to be characterized with respect to pigment formation.

Many *A. fumigatus* polyketide synthase genes have significant homology with polyketide synthase gene of Mycobacterium tuberculosis (30–40%). Co- infection of *A. fumigatus* with *Mycobacterium tuberculosis* is well known as in the case of fungal balls in the post Kochs cavity of the lungs [102]. Genome sequencing information of *Mycobacterium tuberculosis* H37Rv shows the presence of 22 polyketide synthases of type I and type III, out of which three of the PKS enzymes are characterized and reported to be involved in the production of mycolic acids, phthiocerol dimycocerosate, major virulent factors of the *Mycobacterium tuberculosis* [103, 104].

Aflatoxins

Extensively studied biosynthetic gene cluster for synthesis of toxins so far is the gene cluster producing Aflatoxin by *A. parasiticus* and *A. flavus*. The genes encoding the aflatoxin biosynthetic pathway enzymes are clustered within a 75 kb region in *A. flavus* genome [105, 106]. Aflatoxin gene cluster includes *pks*A, a large gene of 6.6 kb encoding

for a polyketide synthase that catalyzes the second step, in which hexanoyl tetrahydroxyl anthrone is converted to norsolorinic acid from the hexanoate [107]. The *pks*A, a non reducing polyketide synthase gene, has SAT, KS, AT, PT, ACP, and TE/CYC domains. *A. nidulans* has homologous PKS enzyme (*pks*A) responsible for synthesis of sterigmatocystin, a known intermediate from the Aflatoxin biosynthesis pathway [108]. Annotation of the *A. flavus* genome indicates the presence of 55 secondary metabolite biosynthetic pathway clusters and highest number of PKS enzymes than any other sequenced *Aspergillus* species. Apart from Aflatoxin, cyclopiazonic acid biosynthetic clusters and genes encoding for Aflatrem have been identified from *A. flavus* [109]. Out of 24 putative PKS enzymes only *aflC* (*pks* A) is the only gene experimentally characterized as a part of Aflatoxin Biosynthetic cluster. Remaining genes still need to be explored with respect to secondary metabolite production. Recently, *A. flavus* secondary metabolite gene clusters were examined for their expression over varied experimental conditions to associate gene clusters with known secondary metabolite functions. These clusters have distinctive gene expression profiles where Aflatoxin and cyclopiazonic (CPA) were found to have unique regulation mechanisms [110]. The fact, that the presence of number of PKS enzymes in *Aspergillus* species exceeds the number of polyketide compounds produced, necessitates further studies of these enzymes using advanced molecular techniques.

Lovastatin

Aspergillus terreus produces non-aromatic, reduced polyketides such as Lovastatin, an HMG-CoA reductase inhibitor. The gene cluster for Lovastatin biosynthesis (over 64 kb) has 18 potential genes as indicated by bioinformatic studies [111]. In the lovastatin pathway, first stable intermediate Dihydromonacolin L is catalyzed by two polyketide synthases LovB and LovF using two acetyl units and eight malonate molecules [112]. LovB/LNKS (lovastatin nonaketide synthase) catalyzes nonketides to a hexahydronaphthalene ring system. The LovB/LNKS, 335 kDa enzyme is a highly reducing iterative type I PKS with KS, AT, DH, MT (ER), KR, and ACP domains. It has a condensation domain (CON) that is similar to the condensation domain of nonribosomal peptide synthetases (NRPSs), which is possibly involved in product transfer or cyclization. It has an inactive ER domain, because of which a separate ER enzyme termed as LovC, carries out the Enoyl reduction function [113]. The other PKS enzyme LovF/LDKS is a simple diketide enzyme, function of which is not characterized fully. The LovF 277 kDa enzyme is similar to LovB and contains domains for KS, AT, DH, ER, and KR. However, its ER domain is active unlike LovB but does not contain the condensation domain (CON). It may encode the enzyme responsible for the biosynthesis of the (2R)-2-methylbutyryl side chain of lovastatin [112, 113]. LovB and LovF lack TE domain required for off loading of growing ketide chain and it is not fully understood how dihydromonacolin L is detached from the enzyme [114]. Lovastatin polyketide synthase (PKS) systems are complex and are of great interest as their genetic engineering can lead to more effective statins with least side effects. Comparative genomic analysis of the Genomes of various *Aspergillus* species can lead to mapping of complex gene machinery for important polyketides.

Recent Approaches to Study Polyketide Synthases for Applications

A number of gene clusters for secondary metabolites are observed to remain silent in various *Aspergillus* species. Lack of studies on the environmental signals that trigger the activation of the gene clusters for the synthesis of polyketides are not well established experimentally. The cryptic gene clusters may code for the biosynthesis of important virulent factors, toxins or even drug candidates. New strategies for their activation are urgently needed to make use of this largely untapped reservoir of the bioactive compounds of *Aspergillus* species. The discovery of new microbial metabolites through genome mining appears to be a promising approach. LaeA, a global regulator of secondary metabolite has proved very helpful in secondary metabolite search [115]. LaeA based gene-mining, deletion and over expression of LaeA in individual *Aspergilli,* can be helpful in deciphering the secondary metabolites genes and their clusters [116]. The terrequinone A gene cluster from *A. nidulans* for asterriquinone biosynthetic pathway, was reported using this approach, combined with microarray analyses [84].

Bioinformatic tools have proved helpful in gaining insight into these megaenzymes which are otherwise difficult to handle experimentally due to their large size and lack of advanced technologies for cloning and expression. Variation of the cumulative GC and window–averaged DNA curvature profile of 26 secondary metabolite gene clusters in *A. fumigatus* genome showed that these clusters are uniquely expressed in *Afu* and may not have been horizontally transferred. Forty percent of secondary metabolite gene clusters with this conserved pattern were related to regulation by LaeA, transcription factor [117]. Recently, phylogenetic studies were performed for type I

polyketide synthases of Ascomycota group fungi, which divides enzymes into eighteen clades indicating the grouping based on presence/absence of reducing and non-reducing domains in PKS, and their linkage with probable polyketide metabolites produced in these fungi [118]. In view of variations in the polyketide biochemical pathways among medically and agriculturally important *Aspergillus* species, phylogenetic relationship can be explored for assigning functionality to unexplored PKS-enzymes with respect to secondary metabolites.

Invasive Aspergillosis: Molecular Diagnostics

Early diagnosis in ABPA and detection of pathogen in invasive Aspergillosis will be of great relevance today. This is particularly in view of the increasing incidence of *Aspergillus* induced Asthma and immunocompromised patients. Often early detection of specific pathogen is hampered due to the lack of specific and sensitive diagnostic methods. In the recent past antifungal drug resistance is often reported. Currently available methods of diagnosis for IA are based on Ag detection and PCR tests. These methods are not satisfactory due to cross reactivity and non specificity. Serological tests based on fungal cell wall components, such as galactomannan or (1,3) β-D-glucan is currently used as a diagnostic tool. However, the incidence of false-positive results of galactomannan-based detection tests is the limitation for this test [119]. PCR methods for *Aspergillus* detection are based on ribosomal DNA (18S rDNA, 28S rDNA, ITS2 and ITS1 regions) but have limitations of genus specificity as they are conserved across a wide range of fungi. PCR based on mycotoxin or polyketide biosynthetic pathway such as melanin or aflatoxin may be of relevance for specific detection of *Aspergilli*. Diversity of the polyketide synthase enzymes in *Aspergillus* species can be explored for detection of important Aspergillus species. Conserved and non conserved regions in the functional domains of polyketide synthase gene can be evaluated for their diagnostic utility. Differential detection of clinically important Aspergillus species, such as *A. fumigatus, A. flavus* and *A. niger* in a multiplex PCR based on gene validations will be of value for invasive Aspergillosis patients. Apart from clinical applications, these tests will also be useful in specific and differential detection of *A. fumigatus, A. flavus* and *A. niger* for the screening of pre and post harvest agricultural products [120, 121].

Aspergillus Research Areas

Current scenario of advancement in the genomic knowledge of *Aspergillus* species can lead to

(i) New Antifungal drug targets
(ii) Novel polyketides of pharmaceutical importance
(iii) Molecular diagnostics for fungal diseases : clinical and agricultural screening
(iv) Design of new antifungal compounds for human health and new polyketides for antifungal treatment
(v) Synthesis of statins with least side effects.

Acknowledgements The facilities and the support provided at Division of Plant Pathology, Indian Agricultural Research Institute by Dr. R.K. Jain, Head of the Department and the Director, IARI are highly acknowledged.

References

1. Tillie-Leblond I, Tonnel AB. Allergic bronchopulmonary aspergillosis. Allergy. 2005;60(8):1004–13. Review.
2. Dagenais TR, Keller NP. Pathogenesis of *Aspergillus fumigatus* in invasive Aspergillosis. Clin Microbiol Rev. 2009;22(3): 447–65. Review.
3. Bennett JW. *Aspergillus*: a primer for the novice. Med Mycol. 2009;47(Suppl 1):S5–12.
4. Karaffa L, Sándor E, Fekete E, Szentirmai A. The biochemistry of citric acid accumulation by *Aspergillus niger*. Acta Microbiol Immunol Hung. 2001;48(3–4):429–40. Review.
5. Barrios-González J, Miranda RU. Biotechnological production and applications of statins. Appl Microbiol Biotechnol. 2010;85 (4):869–83. Review.
6. Cleveland TE, Nierman WC. Genomics of industrial *Aspergilli* and comparison with toxigenic relatives. Food Addit Contam A. 2008;25(9):1147–51.
7. Bhabhra R, Askew DS. Thermotolerance and virulence of *Aspergillus fumigatus*: role of the fungal nucleolus. Med Mycol. 2005;43(Suppl 1):S87–93. Review.
8. Chang YC, Tsai HF, Karos M, Kwon-Chung KJ. THTA, a thermotolerance gene of *Aspergillus fumigatus*. Fungal Genet Biol. 2004;41(9):888–96.
9. Hohl TM, Feldmesser M. *Aspergillus fumigatus*: principles of pathogenesis and host defense. Eukaryot Cell. 2007;6(11): 1953–63.
10. Beauvais A, Monod M, Wyniger J, Debeaupuis JP, Grouzmann E, Brakch N, Svab J, Hovanessian AG, Latgé JP. Dipeptidyl-peptidase IV secreted by *Aspergillus fumigatus*, a fungus pathogenic to humans. Infect Immun. 1997;65(8):3042–7.
11. Shankar J, Nigam S, Saxena S, Madan T, Sarma PU. Identification and assignment of function to the genes of *Aspergillus fumigatus* expressed at 37°C. J Eukaryot Microbiol. 2004;51(4): 428–32.
12. Shankar J, Madan T, Basir SF, Sarma PU. Identification and characterization of polyubiquitin gene from cDNA library of *Aspergillus fumigatus*. Indian J Clin Biochem. 2005;20(1): 208–12.
13. Gautam P, Madan T, Gade WN, Sarma PU. Immunoproteomic analysis of secretory proteins of *Aspergillus fumigatus* with specific Ig E immunoreactivity. Indian J Clin Biochem. 2006;21 (2):12–9.
14. Upadhyay SK, Mahajan L, Ramjee S, Singh Y, Basir SF, Madan T. Expressed sequence tags of *Aspergillus fumigatus*: extension of catalogue and their evaluation as putative drug targets and/or diagnostic markers. Indian J Clin Biochem. 2009;24(2):131–6.

15. Sarma PU, Madan T, Priyadarsini P, Haq W, Katti SB. Polypetides useful for diagnosis of Aspergillus fumigatus and a process for preparing for the same. US patent 6262231.
16. Sarma PU, Madan T, Priyadarsini P, Haq W, Katti SB. A process for the preparation of a novel synthetic peptide epitope useful for diagnosis of aspergillosis. Indian patent 184440.
17. Sarma PU, Madan T, Saxena S. Method of detection of SP-A2 gene variants useful for prediction of predisposition to aspergillosis. US patent 7288376.
18. http://www.allergen.org/search.php?allergensource=Aspergillus+fumigatus.
19. Gautam P, Sundaram CS, Madan T, Gade WN, Shah A, Sirdeshmukh R, Sarma PU. Identification of novel allergens of *Aspergillus fumigatus* using immunoproteomics approach. Clin Exp Allergy. 2007;37(8):1239–49.
20. Sarma PV, Purkayastha S, Madan T, Sarma PU. Expression of an epitopic region of AspfI, an allergen/antigen/cytotoxin of *Aspergillus fumigatus*. Immunol Lett. 1999;70(3):151–5.
21. Madan T, Arora N, Sarma PU. Ribonuclease activity dependent cytotoxicity of Asp fl, a major allergen of *A. fumigatus*. Mol Cell Biochem. 1997;175(1–2):21–7.
22. Saxena S, Madan T, Muralidhar K, Sarma PU. cDNA cloning, expression and characterization of an allergenic L3 ribosomal protein of *Aspergillus fumigatus*. Clin Exp Immunol. 2003;134 (1):86–91.
23. Nigam S, Sarma PV, Ghosh PC, Sarma PU. Characterization of *Aspergillus fumigatus* protein disulfide isomerase family gene. Gene. 2001;281(1–2):143–50.
24. Madan T, Banerjee B, Bhatnagar PK, Shah A, Sarma PU. Identification of 45 kD antigen in immune complexes of patients of allergic bronchopulmonary aspergillosis. Mol Cell Biochem. 1997;166(1–2):111–6.
25. Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P, Denning DW. *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. Microbiology. 2007;153(Pt 6):1677–92.
26. St Leger RJ, Screen SE, Shams-Pirzadeh B. Lack of host specialization in *Aspergillus flavus*. Appl Environ Microbiol. 2000; 66(1):320–4.
27. Mellon JE, Cotty PJ, Dowd MK. *Aspergillus flavus* hydrolases: their roles in pathogenesis and substrate utilization. Appl Microbiol Biotechnol. 2007;77(3):497–504.
28. Madan T, Kishore U, Shah A, Eggleton P, Strong P, Wang JY, Aggrawal SS, Sarma PU, Reid KB. Lung surfactant proteins A and D can inhibit specific IgE binding to the allergens of *Aspergillus fumigatus* and block allergen-induced histamine release from human basophils. Clin Exp Immunol. 1997;110(2): 241–9.
29. Madan T, Eggleton P, Kishore U, Strong P, Aggrawal SS, Sarma PU, Reid KB. Binding of pulmonary surfactant proteins A and D to *Aspergillus fumigatus* conidia enhances phagocytosis and killing by human neutrophils and alveolar macrophages. Infect Immun. 1997;65(8):3171–9.
30. Madan T, Kishore U, Singh M, Strong P, Clark H, Hussain EM, Reid KB, Sarma PU. Surfactant proteins A and D protect mice against pulmonary hypersensitivity induced by *Aspergillus fumigatus* antigens and allergens. J Clin Invest. 2001;107(4): 467–75.
31. Madan T, Kishore U, Singh M, Strong P, Clark H, Hussain EM, Reid KB, Sarma PU. Surfactant proteins A and D protect mice against pulmonary hypersensitivity induced by *Aspergillus fumigatus* antigens and allergens. J Immunol. 2005;174(11): 6943–54.
32. Madan T, Reid KB, Clark H, Singh M, Nayak A, Sarma PU, Hawgood S, Kishore U. Susceptibility of mice genetically deficient in SP-A or SP-D gene to invasive pulmonary aspergillosis. Mol Immunol. 2010;47(10):1923–30.
33. Kaur S, Gupta VK, Thiel S, Sarma PU, Madan T. Protective role of mannan-binding lectin in a murine model of invasive pulmonary aspergillosis. Clin Exp Immunol. 2007;148(2):382–9.
34. Madan T, Kaur S, Saxena S, Singh M, Kishore U, Thiel S, Reid KB, Sarma PU. Role of collectins in innate immunity against aspergillosis. Med Mycol. 2005;43(Suppl 1):S155–63.
35. Madan T, Kaur S, Saxena S, Singh M, Kishore U, Thiel S, Reid KB, Sarma PU. Role of collectins in innate immunity against aspergillosis. Immunobiology. 2002;205(4–5):610–8. Review.
36. Singh M, Madan T, Waters P, Sonar S, Singh SK, Kamran MF, Bernal AL, Sarma PU, Singh VK, Crouch EC, Kishore U. Therapeutic effects of recombinant forms of full-length and truncated human surfactant protein D in a murine model of invasive pulmonary aspergillosis. Mol Immunol. 2009;46 (11–12):2363–9.
37. Mahajan L, Madan T, Kamal N, Singh VK, Sim RB, Telang SD, Ramchand CN, Waters P, Kishore U, Sarma PU. Recombinant surfactant protein-D selectively increase apoptosis in eosinophils of allergic asthmatics and enhances uptake of apoptotic eosinophils by macrophages. Int Immunol. 2008;20(8): 993–1007.
38. Vaid M, Kaur S, Sambatakou H, Madan T, Denning DW, Sarma PU. Distinct alleles of mannose-binding lectin (MBL) and surfactant proteins A (SP-A) in patients with chronic cavitary pulmonary aspergillosis and allergic bronchopulmonary aspergillosis. Clin Chem Lab Med. 2007;45(2):183–6.
39. Upadhyay SK, Mahajan L, Ramjee S, Singh Y, Basir SF, Madan T. Identification and characterization of a laminin-binding protein of Aspergillus fumigatus: extracellular thaumatin domain protein (AfCalAp). J Med Microbiol. 2009;58(Pt 6):714–22.
40. Abad A, Victoria Fernández-Molina J, Bikandi J, Ramírez A, Margareto J, Sendino J, Luis Hernando F, Pontón J, Garaizar J, Rementeria A. What makes *Aspergillus fumigatus* a successful pathogen? Genes and molecules involved in invasive aspergillosis. Rev Iberoam Micol. 2010;27(4):155–82.
41. Rementeria A, López-Molina N, Ludwig A, Vivanco AB, Bikandi J, Pontón J, Garaizar J. Genes and molecules involved in *Aspergillus fumigatus* virulence. Rev Iberoam Micol. 2005;22 (1):1–23.
42. Pihet M, Vandeputte P, Tronchin G, Renier G, Saulnier P, Georgeault S, Mallet R, Chabasse D, Symoens F, Bouchara JP. Melanin is an essential component for the integrity of the cell wall of *Aspergillus fumigatus* conidia. BMC Microbiol. 2009; 9:177.
43. Yu JH, Mah JH, Seo JA. Growth and developmental control in the model and pathogenic aspergilli. Eukaryot Cell. 2006;5(10): 1577–84.
44. Hu W, Sillaots S, Lemieux S, Davison J, Kauffman S, Breton A, Linteau A, Xin C, Bowman J, Becker J, Jiang B, Roemer T. Essential gene identification and drug target prioritization in *Aspergillus fumigatus*. PLoS Pathog. 2007;3(3):e24.
45. Beauvais A, Latgé JP. Membrane and cell wall targets in *Aspergillus fumigatus*. Drug Resist Updat. 2001;4(1):38–49.
46. Chamilos G, Kontoyiannis DP. Update on antifungal drug resistance mechanisms of *Aspergillus fumigatus*. Drug Resist Updat. 2005;8(6):344–58.
47. Latge JP, Mouyna I, Tekaia F, Beauvais A, Debeaupuis JP, Nierman W. Specific molecular features in the organization and biosynthesis of the cell wall of *Aspergillus fumigatus*. Med Mycol. 2005;43:S15–22.
48. Ibrahim-Granet O, Dubourdeau M, Latgé JP, Ave P, Huerre M, Brakhage AA, Brock M. Methylcitrate synthase from *Aspergillus fumigatus* is essential for manifestation of invasive aspergillosis. Cell Microbiol. 2008;10(1):134–48.
49. Maerker C, Rohde M, Brakhage AA, Brock M. Methylcitrate synthase from *Aspergillus fumigatus*. Propionyl-CoA affects

polyketide synthesis, growth and morphology of conidia. FEBS J. 2005;272(14):3615–30.
50. Lorenz MC, Fink GR. The glyoxylate cycle is required for fungal virulence. Nature. 2001;412(6842):83–6.
51. Olivas I, Royuela M, Romero B, Monteiro MC, Mínguez JM, Laborda F, De Lucas JR. Ability to grow on lipids accounts for the fully virulent phenotype in neutropenic mice of *Aspergillus fumigatus* null mutants in the key glyoxylate cycle enzymes. Fungal Genet Biol. 2008;45(1):45–60.
52. Schöbel F, Ibrahim-Granet O, Avé P, Latgé JP, Brakhage AA, Brock M. *Aspergillus fumigatus* does not require fatty acid metabolism via isocitrate lyase for development of invasive aspergillosis. Infect Immun. 2007;75(3):1237–44.
53. Thevelein JM. Regulation of trehalose mobilization in fungi. Microbiol Rev. 1984;48:42–59.
54. Singer MA, Lindquist S. Multiple effects of trehalose on protein folding in vitro and in vivo. Mol Cell. 1998;1:639–48.
55. Al-Bader N, Vanier G, Liu H, Gravelat FN, Urb M, Hoareau CM, Campoli P, Chabot J, Filler SG, Sheppard DC. Role of trehalose biosynthesis in *Aspergillus fumigatus* development, stress response, and virulence. Infect Immun. 2010;78(7): 3007–18.
56. Puttikamonkul S, Willger SD, Grahl N, Perfect JR, Movahed N, Bothner B, Park S, Paderu P, Perlin DS, Cramer RA Jr. Trehalose 6-phosphate phosphatase is required for cell wall integrity and fungal virulence but not trehalose biosynthesis in the human fungal pathogen *Aspergillus fumigatus*. Mol Microbiol. 2010 Jun 9.
57. Schöbel F, Jacobsen ID, Brock M. Evaluation of lysine biosynthesis as an antifungal drug target: biochemical characterization of *Aspergillus fumigatus* homocitrate synthase and virulence studies. Eukaryot Cell. 2010;9(6):878–93.
58. Abadio AK, Kioshima ES, Teixeira MM, Martins NF, Maigret B, Felipe MS. Comparative genomics allowed the identification of drug targets against human fungal pathogens. BMC Genomics. 2011;12(1):75.
59. Goffeau A. Genomics: multiple moulds. Nature. 2005;438(7071): 1092–3.
60. Nierman WC, Pain A, Anderson MJ, Wortman JR, Kim HS, Barrell B, Denning DW. Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. Nature. 2005;438(7071):1151–6.
61. Rokas A, Payne G, Fedorova ND, Baker SE, Machida M, Yu J, Georgianna DR, Dean RA, Bhatnagar D, Cleveland TE, Wortman JR, Maiti R, Joardar V, Amedeo P, Denning DW, Nierman WC. What can comparative genomics tell us about species concepts in the genus *Aspergillus*? Stud Mycol. 2007;59:11–7.
62. Scazzocchio C. Aspergillus genomes: secret sex and the secrets of sex. Trends Genet. 2006;22(10):521–5.
63. Brakhage AA, Schroeckh V. Fungal secondary metabolites—strategies to activate silent gene clusters. Fungal Genet Biol. 2011;48(1):15–22.
64. Khaldi N, Seifuddin FT, Turner G, Haft D, Nierman WC, Wolfe KH, Fedorova ND. SMURF: genomic mapping of fungal secondary metabolite clusters. Fungal Genet Biol. 2010;47(9): 736–41.
65. Gardiner DM, Howlett BJ. Bioinformatic and expression analysis of the putative gliotoxin biosynthetic gene cluster of *Aspergillus fumigatus*. FEMS Microbiol Lett. 2005;248(2): 241–8.
66. Coyle CM, Kenaley SC, Rittenour WR, Panaccione DG. Association of ergot alkaloids with conidiation in *Aspergillus fumigatus*. Mycologia. 2007;99(6):804–11.
67. Maiya S, Grundmann A, Li SM, Turner G. The fumitremorgin gene cluster of *Aspergillus fumigatus*: identification of a gene encoding brevianamide F synthetase. Chembiochem. 2006;7(7): 1062–9.
68. Hissen AH, Wan AN, Warwas ML, Pinto LJ, Moore MM. The *Aspergillus fumigatus* siderophore biosynthetic gene sidA, encoding L-ornithine N5-oxygenase, is required for virulence. Infect Immun. 2005;73(9):5493–503.
69. Lewis RE, Wiederhold NP, Chi J, Han XY, Komanduri KV, Kontoyiannis DP, Prince RA. Detection of gliotoxin in experimental and human aspergillosis. Infect Immun. 2005;73(1): 635–7.
70. Frisvad JC, Rank C, Nielsen KF, Larsen TO. Metabolomics of *Aspergillus fumigatus*. Med Mycol. 2009;47(s1):S53–71.
71. Pel HJ, Visser J, Stam H. Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. Nat Biotechnol. 2007;25(2):221–31.
72. Vala AK, Dave BP, Dube HC. Chemical characterization and quantification of siderophores produced by marine and terrestrial aspergilli. Can J Microbiol. 2006;52(6):603–7.
73. Mogensen JM, Nielsen KF, Samson RA, Frisvad JC, Thrane U. Effect of temperature and water activity on the production of fumonisins by *Aspergillus niger* and different *Fusarium* species. BMC Microbiol. 2009;9:281.
74. Chang PK, Horn BW, Dorner JW. Clustered genes involved in cyclopiazonic acid production are next to the aflatoxin biosynthesis gene cluster in *Aspergillus flavus*. Fungal Genet Biol. 2009;46(2):176–82.
75. Juvvadi PR, Seshime Y, Kitamoto K. Genomics reveals traces of fungal phenylpropanoid-flavonoid metabolic pathway in the filamentous fungus *Aspergillus oryzae*. J Microbiol. 2005;43(6): 475–86.
76. Kiyota T, Hamada R, Sakamoto K, Iwashita K, Yamada O, Mikami S. Aflatoxin non-productivity of *Aspergillus oryzae* caused by loss of function in the aflJ gene product. J Biosci Bioeng. 2011;111(5):512–517.
77. Calvo AM, Wilson RA, Bok JW, Keller NP. Relationship between secondary metabolism and fungal development. Microbiol Mol Biol Rev. 2002;66(3):447–59.
78. Chiang YM, Chang SL, Oakley BR, Wang CC. Recent advances in awakening silent biosynthetic gene clusters and linking orphan clusters to natural products in microorganisms. Curr Opin Chem Biol. 2011;15(1):137–43.
79. Bok JW, Keller NP. LaeA, a regulator of secondary metabolism in *Aspergillus* spp. Eukaryot Cell. 2004;3(2):527–35.
80. Bok JW, Balajee SA, Marr KA, Andes D, Nielsen KF, Frisvad JC, Keller NP. LaeA, a regulator of morphogenetic fungal virulence factors. Eukaryot Cell. 2005;4(9):1574–82.
81. Kale SP, Milde L, Trapp MK, Frisvad JC, Keller NP, Bok JW. Requirement of LaeA for secondary metabolism and sclerotial production in *Aspergillus flavus*. Fungal Genet Biol. 2008;45 (10):1422–9.
82. Bentley R, Bennet JW. Construction polyketides: from collie to combinatorial biosynthesis. Annu Rev Microbiol. 1999;53:411–46.
83. Chiang YM, Szewczyk E, Davidson AD, Keller N, Oakley BR, Wang CC. A gene cluster containing two fungal polyketide synthases encodes the biosynthetic pathway for a polyketide, asperfuranone, in *Aspergillus nidulans*. J Am Chem Soc. 2009;131(8):2965–70.
84. Reyes-Dominguez Y, Bok JW, Berger H, Shwab EK, Basheer A, Gallmetzer A, Scazzocchio C, Keller N, Strauss J. Heterochromatic marks are associated with the repression of secondary metabolism clusters in *Aspergillus nidulans*. Mol Microbiol. 2010;76(6):1376–86.
85. http://www.knowmycotoxins.com/regulations.htm.
86. Zhang W, Tang Y. In vitro analysis of type II polyketide synthase. Methods Enzymol. 2009;459:367–93.

87. Abe I, Morita H. Structure and function of the chalcone synthase superfamily of plant type III polyketide synthases. Nat Prod Rep. 2010;27(6):809–38.
88. Manzoni M, Rollini M. Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs. Appl Microbiol Biotechnol. 2002;58 (5):555–64.
89. Tang Y, Chen AY, Kim CY, Cane DE, Khosla C. Structural and mechanistic analysis of protein interactions in module 3 of the 6-deoxyerythronolide B synthase. Chem Biol. 2007;14(8):931–43.
90. Seshime Y, Juvvadi PR, Fuji I, Kitamoto K. Discovery of a novel superfamily of type III polyketide synthases in *Aspergillus oryzae*. Biochem Biophys Res Commun. 2005;331(1):253–60.
91. Cleveland TE, Yu J, Fedorova N, Bhatnagar D, Payne GA, Nierman WC, Bennett JW. Potential of *Aspergillus flavus* genomics for applications in biotechnology. Trends Biotechnol. 2009;27(3):151–7. Review.
92. Chiang YM, Oakley BR, Keller NP, Wang CC. Unraveling polyketide synthesis in members of the genus *Aspergillus*. Appl Microbiol Biotechnol. 2010;86(6):1719–36.
93. Crawford JM, Thomas PM, Scheerer JR, Vagstad AL, Kelleher NL, Townsend CA. Deconstruction of iterative multidomain polyketide synthase function. Science. 2008;320:243–6.
94. Langfelder K, Jahn B, Gehringer H, Schmidt A, Wanner G, Brakhage AA. Identification of a polyketide synthase gene (pksP) of *Aspergillus fumigatus* involved in conidial pigment biosynthesis and virulence. Med Microbiol Immunol. 1998;187: 79–89.
95. Watanabe A, Fujii I, Tsai HF, Chang YC, Kwon-Chung KJ, Ebizuka Y. *Aspergillus fumigatus* alb1 encodes naphthopyrone synthase when expressed in *Aspergillus oryzae*. FEMS Microbiol Lett. 2000;192:39–44.
96. Jahn B, Langfelder K, Schneider U, Schindel C, Brakhage AA. PKSP-dependent reduction of phagolysosome fusion and intracellular kill of *Aspergillus fumigatus* conidia by human monocyte-derived macrophages. Cell Microbiol. 2002;4:793–803.
97. Langfelder K, Philippe B, Jahn B, Latgé JP, Brakhage AA. Differential expression of the *Aspergillus fumigatus* pksP gene detected in vitro and in vivo with green fluorescent protein. Infect Immun. 2001;69:6411–8.
98. Pihet M, Vandeputte P, Tronchin G, Renier G, Saulnier P, Georgeault S, Mallet R, Chabasse D, Symoens F, Bouchara JP. Melanin is an essential component for the integrity of the cell wall of *Aspergillus fumigatus* conidia. BMC Microbiol. 2009;9:177.
99. Schmaler-Ripcke J, Sugareva V, Gebhardt P, Winkler R, Kniemeyer O, Heinekamp T, Brakhage AA. Production of pyomelanin, a second type of melanin, via the tyrosine degradation pathway in *Aspergillus fumigatus*. Appl Environ Microbiol. 2009;75:493–503.
100. Jørgensen TR, Park J, Arentshorst M, van Welzen AM, Lamers G, Vankuyk PA, Damveld RA, van den Hondel CA, Nielsen KF, Frisvad JC, Ram AF. The molecular and genetic basis of conidial pigmentation in *Aspergillus niger*. Fungal Genet Biol. 2011;48(5):544–53.
101. Jahn B, Boukhallouk F, Lotz J, Langfelder K, Wanner G, Brakhage AA. Interaction of human phagocytes with pigmentless *Aspergillus* conidia. Infect Immun. 2000;68(6):3736–9.
102. Agarwal R, Singh N, Aggarwal AN. An unusual association between *Mycobacterium tuberculosis* and *Aspergillus fumigatus*. Monaldi Arch Chest Dis. 2008;69(1):32–4.
103. Sankaranarayanan R, Saxena P, Marathe UB, Gokhale RS, Shanmugam VM, Rukmini R. A novel tunnel in mycobacterial type III polyketide synthase reveals the structural basis for generating diverse metabolites. Nat Struct Mol Biol. 2004;11(9): 894–900.
104. Chopra T, Gokhale RS. Polyketide versatility in the biosynthesis of complex mycobacterial cell wall lipids. Methods Enzymol. 2009;459:259–94.
105. Payne GA, Brown MP. Genetics and physiology of aflatoxin biosynthesis. Annu Rev Phytopathol. 1998;36:329–62.
106. Yu JJ, Chang PK, Ehrlich KC, et al. Clustered pathway genes in aflatoxin biosynthesis. Appl Environ Microbiol. 2004;70:1253–62.
107. Yabe K, Nakajima H. Enzyme reactions and genes in aflatoxin biosynthesis. Appl Microbiol Biotechnol. 2004;64(6):745–55. Review.
108. Yu JH, Leonard TJ. Sterigmatocystin biosynthesis in *Aspergillus nidulans* requires a novel type I polyketide synthase. J Bacteriol. 1995;177(16):4792–800.
109. Nicholson MJ, Koulman A, Monahan BJ, Pritchard BL, Payne GA, Scott B. Identification of two aflatrem biosynthesis gene loci in *Aspergillus flavus* and metabolic engineering of *Penicillium paxilli* to elucidate their function. Appl Environ Microbiol. 2009;75(23):7469–81.
110. Georgianna DR, Fedorova ND, Burroughs JL, Dolezal AL, Bok JW, Horowitz-Brown S, Woloshuk CP, Yu J, Keller NP, Payne GA. Beyond aflatoxin: four distinct expression patterns and functional roles associated with *Aspergillus flavus* secondary metabolism gene clusters. Mol Plant Pathol. 2010;11(2):213–26.
111. Ma SM, Li JW, Choi JW, Zhou H, Lee KK, Moorthie VA, Xie X, Kealey JT, Da Silva NA, Vederas JC, Tang Y. Complete reconstitution of a highly reducing iterative polyketide synthase. Science. 2009;326(5952):589–92.
112. Hutchinson CR, Kennedy J, Park C, Kendrew S, Auclair K, Vederas J. Aspects of the biosynthesis of non-aromatic fungal polyketides by iterative polyketide synthases. Antonie Van Leeuwenhoek. 2000;78(3–4):287–95. Review.
113. Kennedy J, Auclair K, Kendrew SG, Park C, Vederas JC, Hutchinson CR. Modulation of polyketide synthase activity by accessory proteins during lovastatin biosynthesis. Science. 1999;284(5418):1368–72.
114. Ma SM, Tang Y. Biochemical characterization of the minimal polyketide synthase domains in the lovastatin nonaketide synthase LovB. FEBS J. 2007;274(11):2854–64.
115. Keller NP, Turner G, Bennett JW. Fungal secondary metabolism—from biochemistry to genomics. Nat Rev Microbiol. 2005;3:937–47.
116. Panagiotou G, Andersen MR, Grotkjaer T, Regueira TB, Nielsen J, Olsson L. Studies of the production of fungal polyketides in *Aspergillus nidulans* by using systems biology tools. Appl Environ Microbiol. 2009;75(7):2212–20.
117. Do JH, Miyano S. The GC and window-averaged DNA curvature profile of secondary metabolite gene cluster in *Aspergillus fumigatus* genome. Appl Microbiol Biotechnol. 2008;80(5):841–7.
118. Kroken S, Glass NL, Taylor JW, Yoder OC, Turgeon BG. Phylogenomic analysis of type I polyketide synthase genes in pathogenic and saprobic ascomycetes. Proc Natl Acad Sci USA. 2003;100(26):15670–5.
119. Racil Z, Kocmanova I, Lengerova M, Weinbergerova B, Buresova L, Toskova M, Winterova J, Timilsina S, Rodriguez I, Mayer J. Difficulties in using 1, 3-{beta}-D-glucan as the screening test for the early diagnosis of invasive fungal infections in patients with haematological malignancies–high frequency of false-positive results and their analysis. J Med Microbiol. 2010;59(Pt 9):1016–22.
120. Atoui A, Mathieu F, Lebrihi A. Targeting a polyketide synthase gene for *Aspergillus carbonarius* quantification and ochratoxin A assessment in grapes using real-time PCR. Int J Food Microbiol. 2007;115(3):313–8.
121. Usha Sarma P. Fascinating Potential of *Aspergilli*. Indian J Clin Biochem. 2010;25(4):331–4.