Ind J Clin Biochem (Oct-Dec 2010) 25(4):331–334
DOI 10.1007/s12291-010-0079-x

EDITORIAL

Fascinating Potential of *Aspergilli*

P. Usha Sarma

Published online: 16 November 2010

Aspergillus species are of immense interest to scientific community due to biological, ecological and metabolic diversity. *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* play key role in the clinical manifestation of a spectrum of *Aspergillus* induced diseases both in immunocompetent and immunocompromised hosts. Several novel allergens of *A. fumigatus* are identified and characterized some of which have diagnostic relevance. The role of some of these allergens in immune mechanisms and immunopathogenesis are examined in detail and the host pathogen interactions are well established. *A. oryzae* and *A. niger* are industrially useful in view of their use in the production of soy sauce, citric acid, and enzymes such as glucose oxidase and lysozyme etc. These two species are also used as hosts for production of heterologous proteins of clinical importance. *A. flavus* and *A. parasiticus* produce aflatoxins, and sterigmatocystin that are highly potent, naturally occurring toxins known to humans. Aflatoxin contamination in the agricultural crops and agri-products is an important challenge to the scientists in view of the WTO stipulations. The permissible limit of Aflatoxin is 4–20 ppb in the food and food products in international trade. Several important mycotoxins of *Aspergillus* species include Aflatoxins, Sterigmatocystin, Ochratoxin, Cyclopiazonic Acid, etc. Melanin, a pigment produced by *A. fumigatus* is attributed to the virulence of the pathogen and the disease. It is interesting to note that *A. fumigatus* secretes a variety of toxins such as gliotoxin, an immune suppressor and a protein toxin Aspf1, which is a multifunctional protein exhibiting IgE and IgG inducing properties with ribonuclease activity and cytotoxic properties, besides an important polyketide, melanin.

Recent advances in genomic, proteomic and metabolomic sciences lead to the knowledge on genomic sequences of 15 *Aspergillus* species and identification and characterization of a number of proteins, allergens and genes of important biochemical pathways. This invariably triggered the mining of useful metabolites, novel genes, proteins and secondary metabolites of pharmaceutical, clinical and industrial importance. Comparative functional genomic studies can now be carried out today for *Aspergillus* genus to establish the potential of this genus for its biologically and pharmaceutically important products. Genome size of important *Aspergillus* species varies between 30 and 40 Mb with good synteny between species ($\sim$50%). Currently, it is estimated that approximately more than 30% of genes of *A. fumigatus* have been functionally characterized, while still most of the genes remain unknown with respect to function, possibly a goldmine of biological products useful for human health. Similar status of genes is reported for several other important *Aspergillus* species.

The ability of *Aspergillus* species to produce a broad range of secondary metabolites and polyketides is a challenge to researchers particularly in view of their application value. One of the important applications of polyketide structures namely the Statins, for their cholesterol lowering affects in human health. In 2006, statins led the Forbes magazine' list of America's 20 Best selling drugs, with $8.4 billion and the forecast is an increase in the usage of statins. Aflatoxins are of agricultural importance in view of their high toxicity through contaminated food products which contribute to great economical losses to the country. Melanin, a pigment produced by *A. fumigatus* synthesized by polyketide biosynthetic pathway is a virulence factor of *A. fumigatus* in causing a variety of *Aspergillus* diseases

P. Usha Sarma (✉)
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110012, India
e-mail: pusarma@yahoo.com

and the pathogenesis. It is fascinating to observe that a majority of polyketides in *Aspergillus* species are produced by polyketide biosynthetic pathway. However a great amount of diversity exists in the biochemical reactions and functional aspects of polyketides. One of the key enzymes in the pathway, the polyketide synthase is known to exhibit diversity in domain structures and functions. The key enzyme polyketide synthase is present in all the *Aspergillus* species. Genes responsible for the biosynthesis of secondary metabolites such as aflatoxins are those encoding polyketide synthases, fatty acid synthases, carboxylases, dehydrogenases, reductases, oxidases, oxidoreductases, epoxide hydrolases, mono- or di-oxigenases, cytochrome P450 monooxigenases, and methyltransferases. In the *A. flavus* EST database, numerous genes fall within the categories of these enzymes. Without additional biological evidence it is very difficult to predict whether these genes are involved in primary or secondary metabolism based purely on the bioinformatic annotations only.

Biosynthetic pathway of another important polyketide, the conidial pigment melanin from *A. fumigatus*, a known virulent factor, has also been established. In vitro experiments showed that melanin protects the conidia from phagocytosis and increases their resistance to reactive oxygen species produced by phagocytic cells. Melanin is synthesized through the dihydroxynaphthalene (DHN)-melanin pathway in *A. fumigatus.* Its biosynthesis involves six genes, organized in a cluster, which are expressed during conidiation such as polyketide synthases (polyketide synthase P), hydroxynaphthalene reductase (Ayg1), scytalone dehydratase, Multicopper oxidase, vermelone dehydratase and Laccases. This complex pathway starts with acetyl-CoA and malonyl-CoA converted by polyketide synthase (PksP) (also called ALB1) and AYG1 into 1,3,6,8 tetrahydroxynaphthalene (THN). Then, by successive steps of reduction and dehydration, THN is converted to 1,8-DHN, which is finally polymerized by fungal laccases to melanins.

Polyketide synthases are multidomain and multifunctional enzymes with 1,600–2,000 AA. Functions of polyketide synthases are also found to be associated with non-ribosomal peptide synthases. Fungal polyketide synthases are different compared to bacterial enzymes in terms of their structure as well as conformational domain organization. Fungal Polyketide synthases are of type I polyketide synthases which use their domain repeatedly while bacterial polyketide synthases are of Type I, II and Type III polyketide synthases and have modular domain structures, used once or repeatedly. The crystal structure of polyketide synthase of *Aspergillus* species is not yet established. The crystal structures of 6-deoxyerythronolide B synthase, a microbial polyketide synthase from Streptomyces and a type III Polyketide synthases from *Mycobacterium tuberculosis* are available. This facilitated understanding on metabolite diversity based on protein architecture in nature.

In order to exploit the bioresource potential of *Aspergillus* species, particularly for secondary metabolites like polyketides, it is necessary to examine the diversity of polyketide synthases, in different *Aspergilli* with respect to the novel polyketides of importance. In this context bioinformatics and molecular biology tools are of immense value, particularly with the availability of genomic sequences of *Aspergillus* species even for a comparative genomic analysis. The reported Polyketide synthases in Aspergillus species are: 24 in *A. fumigatus*, 25 in *A. flavus*, 46 in *A. niger* and 17 in *A. nidulans*. However based on the, conserved motifs of Ketosynthase domain, lesser number of polyketide synthases such as 14 from *A. fumigatus*, 23 in *A. flavus*, 32 in *A. niger*, and 27 in *A. oryzae* (unpublished data) are identified. They contain essential domains such as Keto synthase (KS), Acyl carrier protein (ACP), and acyl transferase (AT), while Methyltransferase (MT), dehydrogenase (DH) and enoyl reductase (ER) are variable depending on the type of polyketide synthases. Based on the absence and presence of MT, DH and ER domain, Polyketide synthases are classified as non reducing (NR) and reducing Polyketide synthases [partially reducing (PR) and highly reducing (HR)]. This classification facilitates to determine the type of compounds they can produce. Moreover, SAT (starter unit-ACP transacylase) and PT (product template) domains that control the structural outcome of NR-polyketide synthases were recently identified. Non-reduced Polyketide synthase basically produces compounds with no reduction reactions, as in the case of Aflatoxins, Melanin pigments etc. While reducing polyketide synthases have domains for reduction such DH, MT and ER, which produce highly reduced compounds such as 6 methylsalicylic acid and lovastatins.

Recently a novel global regulator of secondary metabolite LaeA, a nuclear protein in *Aspergilli* was reported. In up regulation of the terrequinone A, an antimicrobial from *A. nidulans*, LaeA regulation occurs at transcriptional level and has high homology with methyl transferase, which is known to regulate gene expression by modifying chromatin structure. Deletion and over expression of LaeA in individual *Aspergilli*, has proved helpful in deciphering the secondary metabolite genes and their clusters as in terriquinone biosynthetic pathway. Same approach can be applied for other *Aspergilli* like *A. fumigatus* and *A. oryzae* where single copy of LaeA is reported. Combination of bioinformatic tools and degenerate primer based or LaeA based gene mining will help to identify and characterize repertoire of polyketide synthases in *Aspergillus*. Most of the polyketide synthase clusters are silent in in vitro conditions. The key to activate such cryptic polyketide

synthase clusters for the identification of novel polyketides of medical and pharmaceutical importance is the need of the day. An alternate approach using bioinformatic tools Udwary-Merski algorithm (UMA) and functional analysis will be of use for possible engineering and production of polyketides with desired functions for human health.

Diversity of the polyketide synthase enzymes in *Aspergillus* species is a challenge to be addressed as this leads to the production of variety of diverse products such as lovastatins, aflatoxins, and melanin etc. It is not far off to get more effective, versatile and safer statins such as lovastatin in view of better understanding of Apergillus genomics. Melanins can be of relevance as the pathway is well understood in many fungi of medical and agricultural importance. Chemo informatics on secondary metabolites of *Aspergillus* species facilitates designing the inhibitors targeting the pigments, produced at the time of germination. Such inhibitors can curtail the growth of fungi at early stage. An example is tricyclazole, a fungicide that specifically inhibits hydroxynaphthalene reductase involved in DHN-melanin biosynthesis. Advances in Aspergillus genomics and research today opened up great opportunity to understand the biological and biochemical mechanism. The potential of *Aspergilli* for synthesis of novel polyketide for human health can be maximized.

References

1. Dagenais TR, Keller NP. Pathogenesis of *Aspergillus fumigatus* in invasive Aspergillosis. Clin Microbiol Rev. 2009;22(3): 447–65.
2. Priyadarsiny P, Swain PK, Sarma PU. Expression and characterization of Asp f1, an immunodominant allergen/antigen of *A. fumigatus* in insect cell. Mol Cell Biochem. 2003;252(1–2): 157–63.
3. 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.
4. Shankar J, Nigam S, Saxena S, Madan T, Sarma PU. Identification and assignment of function to the genes of *Aspergillus fumigatus* expressed at 37 degrees C. J Eukaryot Microbiol. 2004;51(4):428–32.
5. 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 increases apoptosis in eosinophils of allergic asthmatics and enhances uptake of apoptotic eosinophils by macrophages. Int Immunol. 2008;20(8):993–1007.
6. Madan T, Kishore U, Singh M, Strong P, Hussain EM, Reid KB, Sarma PU. Protective role of lung surfactant protein D in a murine model of invasive pulmonary aspergillosis. Infect Immun. 2001;69(4):2728–31.
7. 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.
8. Madan T, Reid KB, Singh M, Sarma PU, Kishore U. Susceptibility of mice genetically deficient in the surfactant protein (SP)-A or SP-D gene to pulmonary hypersensitivity induced by antigens and allergens of *Aspergillus fumigatus*. J Immunol. 2005;174(11):6943–54.
9. Machida M, Terabayashi Y, Sano M, Yamane N, Tamano K, Payne GA, Yu J, Cleveland TE, Nierman WC. Genomics of industrial Aspergilli and comparison with toxigenic relatives. Food Addit Contam A Chem Anal Control Expo Risk Assess. 2008;25(9):1147–51.
10. Bennett JW. *Aspergillus*: a primer for the novice. Med Mycol. 2009;47 Suppl 1:S5–12.
11. Reverberi M, Ricelli A, Zjalic S, Fabbri AA, Fanelli C. Natural functions of mycotoxins and control of their biosynthesis in fungi. Appl Microbiol Biotechnol. 2010;87(3):899–911. Epub Accessed 22 May 2010.
12. Madan T, Priyadarsiny P, Vaid M, Kamal N, Shah A, Haq W, Katti SB, Sarma PU. Use of a synthetic peptide epitope of Asp f 1, a major allergen or antigen of *Aspergillus fumigatus*, for improved immunodiagnosis of allergic bronchopulmonary aspergillosis. Clin Diagn Lab Immunol. 2004;11(3):552–8.
13. Toyotome T, Watanabe A, Iwasaki A, Kamei K. Strategy of *Aspergillus fumigatus* to evade attacks from host—projectile weapons and armor. Nippon Ishinkin Gakkai Zasshi. 2009;50(3): 139–45.
14. Archer DB, Dyer PS. From genomics to post-genomics in *Aspergillus*. Curr Opin Microbiol. 2004;7(5):499–504.
15. Jones MG. The first filamentous fungal genome sequences: *Aspergillus* leads the way for essential everyday resources or dusty museum specimens? Microbiology. 2007;153(1):1–6.
16. Barrios-González J, Miranda RU. Biotechnological production and applications of statins. Appl Microbiol Biotechnol 2010; 85(4):869–83. Epub Accessed 10 Oct 2009.
17. Yu J, Whitelaw CA, Nierman WC, Bhatnagar D, Cleveland TE. *Aspergillus flavus* expressed sequence tags for identification of genes with putative roles in aflatoxin contamination of crops. FEMS Microbiol Lett. 2004;237(2):333–40.
18. Tsai HF, Chang YC, Washburn RG, Wheeler MH, Kwon-Chung KJ. The developmentally regulated ALB1 gene of *Aspergillus fumigatus*: its role in modulation of conidial morphology and virulence. J Bacteriol. 1998;180:3031–8.
19. 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(12):793–803.
20. 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: 177–189.
21. 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. Epub Accessed 2 Apr 2010.
22. 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. Epub Accessed 1 Aug 2004.
23. 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.
24. Sanchez JF, Chiang YM, Wang CC. Diversity of polyketide synthases found in the *Aspergillus* and Streptomyces genomes. Mol Pharm. 2008;5(2):226–33. Epub Accessed 14 Mar 2008.

25. Pain A, Böhme U, Berriman M. Genome watch: hot and sexy moulds!. Nat Rev Microbiol. 2006;4:244–5.
26. Crawford JM, Thomas PM, Scheerer JR, Vagstad AL, Kelleher NL, Townsend CA. Deconstruction of iterative multidomain polyketide synthase function. Science. 2008;320:243–6.
27. Keller NP, Turner G, Bennett JW. Fungal secondary metabolism—from biochemistry to genomics. Nat Rev Microbiol. 2005;3:937–47.
28. Perrin RM, Fedorova ND, Bok JW, Cramer RA, Wortman JR, Kim HS, Nierman WC, Keller NP. Transcriptional regulation of chemical diversity in *Aspergillus fumigatus* by LaeA. PLoS Pathog. 2007;3(4).
29. Bok JW, Hoffmeister D, Maggio-Hall LA, Murillo R, Glasner JD, Keller NP. Genomic mining for *Aspergillus* natural products. Chem Biol. 2006;13(1):31–7.
30. Udwary DW, Merski M, Townsend CA. A method for prediction of the locations of linker regions within large multifunctional proteins, and application to a type I polyketide synthase. J Mol Biol. 2002;323(3):585–98.
31. 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. Epub Accessed 23 Jan 2009.

Websites cited

1. http://www.knowmycotoxins.com/regulations.htm.
2. Immunoepitope database and analysis resource. http://immuneepitope.org/refid/511.