International Journal of Microbiological Research 6 (1): 34-41, 2015
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2015.6.1.92217

Genomic and Proteomic Properties of Aspergillus Species: The Prospects and the Breakthrough

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Abstract: The aspergilli are a fascinating group of fungi known for their ubiquitous significance in medicine, agriculture, industry as well as in the environment. However, its detrimental effects is on the upsurge thereby requiring deep understanding of this at the –omics level. This review shows that protein level analysis would be important to understand the detail of the organisms as it allows for location specific analysis, study of post translational modification such as glycosylation and phosphorylation which might even impact signal transduction. In another dimension, proteomic analysis provides systematic perspective on fungal physiology and has been used in various studies to elucidate protein associated with osmoadaptation in aspergilli as well as deciphering their virulence factors. At the genomic level, evidence that there is no similarity in percentage of genome identity and lifestyle of organisms was found, sexual cycle and mating gene has been confirmed in this genus in addition to other important observations in the review. It can therefore be concluded, that better understanding of the genus aspergilli and other fungal genera is central to how much of the field of genomics and proteomics is known.

Key words: Genomics · Proteomics · Aspergillus

INTRODUCTION

The aspergilli are a fascinating group of fungi exhibiting immense ecological and metabolic diversity [1]. These organisms have relevance significance in agriculture, medicine biotechnology as well as in the environment [2-4]. In agriculture, production of mycotoxins is one of the notable effects of these fungal genera as they are involve in contamination of both pre and post harvest commodities including the ready to eat foods [5, 6, 9], thereby reducing the nutritional composition as well the technological quality of the infected foods [7-8]. Evidence from research findings shows that most of the toxins produce by the Aspergillus genera are hepatotoxic, immunotoxic, carcinogenic, nephrotoxic and has even been implicated in various other disorders [10-13]. In medicine, their role in the etiology of invasive aspergillosis has been documented [14]. Among the human pathogenic species of Aspergillus, *A. fumigatus* is the primary causative agent of human infections, followed by *A. flavus*, *A. terreus*, *A. niger* and the model organism, *A. nidulans* [15-16]. Aspergilli cause a wide range of human ailments depending on the immune status of the host [15,17]. In individuals with altered lung function such as asthma and cystic fibrosis patients, aspergilli can cause allergic bronchopulmonary aspergillosis, a hypersensitive response to fungal components. Noninvasive aspergillomas may form following repeated exposure to conidia and target preexisting lung cavities such as the healed lesions in tuberculosis patients. Invasive aspergillosis (IA) is perhaps the most devastating of Aspergillus-related diseases, targeting severely immunocompromised patients. Those most at risk for this life-threatening disease are individuals with hematological malignancies such as leukemia; solid-organ and hematopoietic stem cell transplant patients; patients on prolonged corticosteroid therapy, which is commonly utilized for the prevention and/or treatment of graft-versus-host disease in transplant patients; individuals with genetic immunodeficiency such as chronic granulomatous disease (CGD); and individuals infected with human immunodeficiency virus [15,18]. In the biotechnology industry, they have been used to produce a wide

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variety of products ranging from human therapeutics (e.g. antibacterial and antifungal agents) to specialty chemicals (e.g. commercial enzymes, organic acids), which together represent billions of dollars in annual sales [2]. Just one class of compounds, the cholesterol-lowering statins, represents a market of almost US $15 billion per year in the USA [2,3].Not too long, these organisms have received much public interest in the USA and in Denmark owing, respectively, to their prevalent infestation in buildings affected by Hurricane Katrina [4] and in schools affected by repeated flooding, raising health concerns for both adults and school children [19-20]. Owing to these, it thus become imperative to do a mini review on the genomic and proteomic properties of aspergillus species and the impacts of these –omics studies on the understanding of their role in the etiology of hazards in agriculture, medicine, environment as well as their exploitation in the biotechnology industry.

Genomic Properties of *Aspergillus* Species: The genus Aspergillus is characterized by remarkable genome sequence diversity; using proteome divergence as a yardstick. They are also as diverse as our own phylum, the vertebrates, whereas the 'very close' relatives *A.fumigatus* and *Aspergillus fischerianus* are as divergent as humans and mice [21-22]. In contrast to several other fungal lineages where genome structure within and between species is plastic [23] and contrary to what would be expected based on the degree of sequence diversity in the genus, the structure of the *Aspergillus* genomes appears rather stable. All Aspergillus genomes sequenced so far have eight chromosomes, ranging in size from 28 to 40 Mb and appear to have similar characteristics, although karyotype analyses suggest that natural populations of several of these species harbor chromosomal variants [24]. The apparent lack of genome plasticity does not mean that the Aspergillus genome is devoid of conundrums. One question that has attracted considerable interest is why the genomes of species like *A. oryzae* and *A. flavus* are 20% bigger and substantially more gene rich than those of *A. nidulans* and *A. fumigatus*. Several potential explanations could account for the difference, including genome duplication, segmental duplication, as well as massive horizontal gene transfer (HGT). However, an in-depth comparison of the *A. oryzae*, *A. nidulans* and *A. flavus* genomes by Khaldi and coworkers did not find support for any of these explanations [25], suggesting that several different mechanisms, each acting in a piece meal fashion, are likely to account for the difference. Nevertheless, both of these study as well as a few others have provided several examples indicating that the *Aspergillus* genome has been sculpted by HGT serving both as the donor lineage [26] as well as the recipient [25]. Early analysis of the *Aspergillus* genome focused considerably on whether all species have a sexual cycle [27], greatly contributing to what has been termed the 'fungal sexual revolution' [28]. This revolution encompasses not only the demonstration of sex in a few, previously thought to be asexual, species [29], but also the realization that experiments, such as the ability of the mating genes to regulate expression of downstream genes in a mating type specific manner [30], suggest that most, if not all, asexual fungal species have cryptic sexual cycles yet to be discovered. In *A. fumigatus*, perhaps the most celebrated case of fungal sex cycle discovery [31], mixing and matching of pairs has revealed considerable variation in fertility [32], opening the door to understanding why the sexual cycle has been so elusive. Another major and perhaps more complex, question whose investigation has been dramatically enhanced by the availability of genomes is whether Aspergillus populations are genetically differentiated and the implication of population structure for their lifestyles. Global surveys from a variety of species show lack of genetic differentiation [33], however, the presence of distinct lineages in phylogenetic analyses of such cosmopolitan species, which are usually interpreted to represent cryptic species [34], could also be interpreted as evidence for the existence of genetically distinct populations within species. Nevertheless, local examinations often identify considerable levels of differentiation and the existence of genetically distinct populations. For example, despite the lack of differentiation of *A. fumigatus* isolates across the globe [34], a recent analysis of 255 Dutch isolates using data from 20 molecular markers identified five distinct populations (Klaassen *et al.,* 2012). Interestingly, all multidrug-resistant isolates nest within a single, predominantly asexual population, suggesting that both genetic differentiation and reproductive mode influence the dynamics of drug resistance patterns in natural *A. fumigatus* populations [35]. Similarly, the apparent lack of structure in *A. flavus* isolates from around the globe [36], contrasts with the existence and long-term (10 000 years) maintenance of three genetically distinct sympatric populations [37]. Intriguingly, it was showed that crosses of isolates from distinct *A. flavus* populations not only interbreed in the laboratory, but also recombine and convert non aflatoxigenic isolates into aflatoxin-producing ones [38]. Whether and how these laboratory

based findings impact the efficacy of non aflatoxigenic biocontrol strains aiming to competitively exclude their aflatoxin-producing relatives in the field is a major yet unanswered riddle [39]. This lack of association between lifestyle and evolutionary affinity is probably because many of the traits render fungi into potent pathogens, agricultural pests, or cell factories, are generally associated with the saprophytic lifestyle and selected for survival in conditions independent of their current roles in pathogenesis, pestilence, or biotechnology. Although no database contains all 14 available *Aspergillus* genomes, most are available including the Aspergillus Genome Database (Asp GD) [40], Fungi DB [41], Central Aspergillus Data REpository (CADRE) and the Aspergillus Comparative Database (http://www. Broad institute.org/annotation/genome/aspergillus_group/),with the rest available from Gen Bank [42] or the JGI genome portal [43]. Unfortunately, the annotations of some species, such as *A. sojae* [44], have not yet been made publicly available effectively stymieing easy access to some of the data for inclusion in '-omics' studies. Furthermore, the gene models for the 14 currently available genomes have been constructed using several different algorithms and different standards of analysis, which is problematic because the process of whole genome annotation is highly sensitive to the algorithms and assumptions used in constructing these gene models. Take for example, the genomes of two *A. niger* isolates: isolate CBS 513.88 is reported to contain 14 165 genes and isolate ATCC 1015 to contain 11 200 genes, but further analysis suggests that less than one third of the 3000 gene differential between the two annotations is real [45]. This lack of consistency and uniformity in the annotation of Aspergillus genomes significantly reduces the utility and value of the data. For gene-centered studies, elucidation of whether the annotation differences observed at a particular locus across genomes are real is non-trivial, whereas for genome-wide studies, gene number over estimation or underestimation makes studies that fundamentally rely on accurate gene counts, such as examination of gene family evolution or of genome size differences, vulnerable to annotation bias. Although the availability and quality of Aspergillus genome data is a long-standing problem that is unlikely to disappear soon, the advent of next-generation sequencing technologies (NGSTs) have ameliorated another problem [46], namely the ability to generate genomic or other high-throughput sequencing data from any Aspergillus species. Besides the use of NGSTs to sequence the genomes of some of the species of Aspergillus such as *A. kawachii* [47] and *A. sojae* [44], NGSTs have been used to sequence the genomes of additional isolates from already sequenced species [48], whereas NGST applications such as RNA-Seq [46], have been employed to characterize the structure and variation of the Aspergillus transcriptome [49-50]. For example, in the most thorough application of these technologies in the genus Aspergillus to date, Gibbons and coworkers sequenced the genomes of seven *A.oryzae* and seven *A. flavus* isolates as well as three of the transcriptomes of each species [48]

Proteomics Characteristics of *Aspergillus* Species: It is true that protein-level analysis is particularly relevant in eukaryotic systems, such as fungi because it allows location-specific analysis as well as the study of post translational modifications (e.g.phosphorylation, glycosylation), which might impact on phenomena such as signal transduction [51]. One of the earliest intracellular filamentous fungal proteomic studies was performed by Herna´ndez-Macedo *et al.* [52] on the wood-degrading fungi viz ; *P. chrysosporium* and *Lentinula edodes* using 2DE to conduct a differential comparison of cytoplasmic protein expression patterns in the presence or absence of iron. They visualized 21 proteins related to iron uptake in these ligninolytic fungi. However, the subsequent identification of these proteins was deficient and therefore Grinyer *et al*. [53] provided further Progress in fungal proteomics by using mass spectrometry (both MALDI-TOF and LC-MS/MS) to identify proteins from *T.harzianum* whole-cell protein extract. Of the hundreds of proteins resolved in a single gel, the researchers identified 25 (out of 96 attempted) to provide an initial proteome map. Although this identification approach has been commonly used in proteomic studies of other organism [54]. Grinyer *et al.* [55] were the first to use it to study filamentous fungi. Building on this established approach, the researchers further studied the differential whole-cell proteome, as well as the secretome (proteome of secreted proteins), of *T.atroviride* grown in media containing either *Rhizoctonia solani* cell walls or glucose [55]. The researchers identified 24 protein spots, which contained both previously known cell wall-degrading enzymes and previously uncharacterized novel proteases. Several proteomic studies have begun to appear in the literature for the genus *Aspergillus*. Melin and colleagues provided the first protein identifications for the *A. nidulans* proteomes [56,57]. They co-cultivated the fungus with a lactic acid-producing bacteria and showed specific changes in protein expression levels that correlated with the morphological changes caused by the

co-cultivation. Two other reports characterized the proteome of *A. fumigatus*, perhaps the most prominent filamentous fungal human pathogen [58]. The first *A. fumigatus* proteome map was provided by Kniemeyer *et al.* [59] who conducted a systematic characterization of carbon catabolite repression by comparing protein expression patterns during growth on two different carbon sources. For growth on ethanol, 52 proteins were identified, for which many key gluconeogenesis, glyoxylate cycle and ethanol degradation enzymes were found to be up-regulated. Later, Carberry *et al.* [60] added 28 additional protein identifications to the *A. fumigatus* proteome map, showing for the first time that the eukaryotic elongation factor 1Bg protein exhibits glutathione transferase activity. This latter example illustrates that proteomic analysis not only provides a systematic perspective on fungal physiology, but also serves as an hypothesis-generating tool. Kim *et al.* [61] further observed a similar benefit when they recently updated the *A. nidulans* proteome map with identification of 30 additional proteins, including five that were not characterized and were found to be involved in osmoadaptation. Proteomic analysis is also being used to develop systematic understanding of virulence factors in pathogenic fungi. For example, Ferna´ndez-Acero *et al.* [62] who provided the first proteome 2DE map of *Botrytis cinerea*, found that many of the identified proteins were isoforms of malate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase, correlating them to the phytopathogenic nature of *B. cinerea*. The authors followed up their first study by identifying pathogenicity factors and therapeutic targets for *B. cinerea* [63]. Similarly, the proteome of another phytopathogenic fungus, *S. sclerotiorum*, was recently mapped [63] and provided clues that α-L-arabinofuranosidase might be involved in the pathogenicity of the fungus. Proteomic analysis in fungi is also providing insight related to systematic metabolic flux changes. Shimizu *et al.* [63] resolved 1100 intracellular and 300 mitochondrial proteins of *P.chrysosporium* in 2D gels and observed that 47 intracellular and 10 mitochondrial proteins were differentially expressed when grown in the presence of vanillin. Their study not only identified key enzymes involved in vanillin metabolism, but also showed *P. chrysosporium's* metabolic shift from glyoxylate cycle to the tricarboxylic acid cycle. Similarly, their work showed that *A. nidulans* shifts metabolic flux toward glycerol biosynthesis during osmoadaptation and has reduced expression of pathways that are downstream to the tricarboxylic acid cycle (e.g. lysine biosynthesis) and potentially has an increased protein turnover, as evidenced by increased expression of heat shock proteins and Shp1-like protein degradation protein. These studies demonstrate the capacity of proteomics to characterize systematically the various biochemical pathways that might be involved in adaptation to changing environments [63] . Herna´ndez-Macedo *et al.* [52] described procedures for plasma membrane and outer membrane protein extraction of *P. chrysosporium* and *L. edodes*, although the proteins were only visualized in one-dimensional SDS–PAGE rather than 2DE. Later, Aimaniannda *et al.* [64] provided the first subproteome map of *A. fumigatus* surface proteins, with the goal of finding potential therapeutic targets against this human pathogen. It is likely that future cell wall and membrane sub proteomics studies will provide a systematic understanding of proteins involved in both protein secretion and in cell-to-cell interaction during pathogenesis. Mitochondria have also received attention. Grinyer *et al.* [55] were the first to publish a mitochondrial subproteome describing a successful sample preparation protocol and mitochondrial proteome map for *T. harzianum*. Based on protein databases of *N. crassa*, *A. nidulans, A. oryzae*, *S.cerevisiae* and *Schizosaccharomyces pombe*, they identified 25 unique mitochondrial proteins involved in the tricarboxylic acid cycle, chaperones, protein-binding and transport proteins, as well as mitochondrial integral membrane proteins. More recently, Schmitt *et al.* [65] reported on proteomic analysis of the mitochondrial outer membrane of *N. crassa*. The researchers employed LC-MS/MS and MALDI-TOF from 1-D SDS–PAGE to circumvent the difficulty of solubilizing and focusing hydrophobic mitochondrial membrane proteins for 2DE. They were able to identify 30 proteins, of which some are known to be involved in transport (import machinery and transporters) and overall mitochondrial morphology. Most recently, Grinyer *et al.* [55] separated and identified 13 of the 14 subunits of the *T. reesei* 20S proteasome, providing the first filamentous fungal proteasome proteomics. These reports imply that systematic, whole- and even sub-organelle proteomics is possible once adequate organelle separation protocols are in place. The advantage of a sub proteomic approach is that it enables the protein expression to be localized in a particular organelle, thereby providing additional insight into the function of the protein in the given physiological state of the cell. Many of these proteins are of special interest in the study of pathogens [65,66] or during production of recombinant proteins in the biotechnology industry [66].

As a result, a significant number of publications have described the fungal secretome. This might also be owing to the fact that secretome sample preparation is much faster and simpler than extraction and preparation of intracellular proteins. Wilson Francisco and colleagues provided pioneering contributions to this field, establishing a sample preparation protocol for the fungal secretome [67]. Using this protocol, they studied *A. flavus* and identified 22 secreted proteins involved in rutin degradation [67]. This helped develop an initial understanding of the enzymes involved in degradation of secondary metabolites for cellular consumption. They continued this work using LC-MS/MS to identify an additional 51 secreted proteins, of which 18 were found to be in the rutin degradation pathway [68]. Oda *et al.* [69] studied the secretome of *A. oryzae* and identified 29 extracellular proteins when fungi were grown in either liquid or solid-state culture. Several of the identified proteins were sequestered in cell walls during liquid culture but passed through the cell wall during solid-state growth. Sua´rez *et al.* [70] studied the secretome of *T.harzianum* grown using either chitin (a key cell wall component) or the actual cell walls of other fungi (*R. solani, B. cinerea, or Pythiumultimum*) as a nutrient source. For each different substrate, they found significant differences in 2DE maps of extracellular proteins. However, despite these differences, the most abundant protein under all conditions was a novel aspartic protease (P6281), which showed strong homology with polyporopepsin from *Irpex lacteus*. This led to speculation that this protein has a fundamental role in the parasitic activity of Trichoderma spp. Similarly, Marra *et al.* [71] provided a novel proteomic study of three-way interaction between *T. atroviride, R. solani and B. cinerea.* They identified numerous proteins involved in multiple-species cross-talk, providing insight to the host pathogen interaction in nature, as well as to proteins that are potentially specific to pathogenesis. A similar approach was used by Zorn *et al.* [72] in which the secretome of *Pleurotus sapidus* grown on peanut shells was observed. The researchers found that most secreted proteins had acidic isoelectric points (pIs) and there were various metallopeptidases and serine proteases. In an alternative completely non-gel based approach, Dan Cullen and colleagues provided a comprehensive identification of the *P. chrysosporium* secretome using a combination of shotgun LC-MS/MS and database prediction [73]. In conclusion, the genomic and proteomic studies can give an integrated and holistic view of the cell. Also, these field can be used to monitor or modify organisms in a comprehensive way. This is because the emerging synergy between the substantial and ever-expanding bodies of knowledge on Aspergillus genomics, natural history, systematics, molecular genetics and development, natural products chemistry, human, animal, plant disease and biotechnology, coupled with the remarkable phenotypic versatility present in the genus, make it ideal for addressing fundamental questions across several levels of biological organization. It is thus imperative that in near future, we would be able to understand and provide insight and explicit explanation for the molecular mechanisms behind the cryptic nature of sexual cycle in the various fungal species, understand how both primary and secondary metabolites are produced at the molecular level as well as the molecular basis of certain fungal pathogenicity in both plants and animals among other unclear facts.

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