Aspergilli: Models for systems biology in filamentous fungi

Brandl, Julian; Andersen, Mikael Rørdam

Published in:
Current Opinion in Systems Biology

Link to article, DOI:
10.1016/j.coisb.2017.09.005

Publication date:
2017

Document Version
Peer reviewed version

Aspergilli: Models for Systems Biology in Filamentous Fungi

Julian Brandl[a], Mikael R. Andersen[a,*]

[a]*Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs. Lyngby, Denmark*

Abstract

Aspergillus is a diverse genus of filamentous fungi including common house hold mold as well as human pathogens. More than 350 species are currently part of this genus and all their genomes are soon to be sequenced. The availability of this vast amount of data will allow for more in-depth understanding of genetic traits governing desirable properties like enzyme production as well as the pathogenic potency of the organisms. In this review we give an overview of the systems biology research conducted in Aspergilli. This research has covered omics technologies like genomics, transcriptomics and proteomics where outstanding contributions are highlighted. From past developments it becomes apparent that CRISPR technology will speed up genetic research in the Aspergillus field. This speed up will allow for an increase in systems biology targeted research by accelerating data generation. The increase in throughput of data generation both per experiment and per time will lead to future challenges in the data handling, integration and interpretation.

Keywords: Aspergillus, Systems Biology, Genomics, Proteomics, Transcriptomics, Genome-scale modeling

1. Introduction

The genus Aspergillus comprises more than 350 known species of filamentous fungi[1] exhibiting a great variation in lifestyle (e.g. habitat, pathogenicity) and metabolic proper- ties. Probably the best known representative of this genus is *Aspergillus nidulans*, a genetic model organism that has been extensively studied with respect to metabolism, cellular development, and regulation. The ability to genetically manipulate *A. nidulans* and combine genetic traits by sexual crossing has been crucial for its pronounced role as genetic model organism.

Besides being used as genetic model organisms for filamentous fungi and eukaryotes in general, other species in the genus are known for their occurrence as mould in buildings as well as on food products. *Aspergillus niger* is a very common food spoilage fungus which can be explained by the saprophytic lifestyle endowing *A. niger* with a large repertoire of plant

*Corresponding author

Email addresses: jubra@bio.dtu.dk (Julian Brandl), mr@bio.dtu.dk (Mikael R. Andersen)

URL: staff.dtu.dk/miran (Mikael R. Andersen)

biomass degrading enzymes[2]. While this arsenal enables the undesired growth of *A. niger* on food, those enzymes are at the same time interesting compounds for biotechnological production[3]. Some of the most prominent enzymes produced in *A. niger* are glucose oxidase — a common enzyme for the quantification of blood sugar — and α-amylase that is widely used in the processing of starch in the food industry. *A. niger* is furthermore well known for its ability to accumulate organic acids, most prominently citric acid in very high amounts[4]. Another member of this genus — *Aspergillus oryzae* — has also been used for centuries in the production of soy sauce and has consequently resulted in *A. niger* and *A. oryzae* as Generally Recognized as Safe (GRAS) an important label for the biotechnological production of food components[5]. This label combined with the high productivity in both organisms has established those fungi as industrial work horses for enzyme production of fungal origin.

In addition to these biotech-relevant representatives of the genus, there are other species that are mostly known for being pathogens to humans (*A. flavus* and *A. fumigatus*) causing aspergillosis in immunocompromised patients resulting in difficult and potentially life-threatening infections[6] or plants (*A. flavus* and *A. niger*), where the fungus spoils crops on the field or post-harvest. Having this wide variety of organisms that are of relevance for humans with regard to health and biotechnological applications sparked an early interest in conducting research on a systems level in many of those fungi. For these reasons — and due to the diverse and active *Aspergillus* research community — the genus has become a model genus for many types of studies. In particular, the advent of genome sequencing has paved the way for the application of several -omics technologies for the elucidation of metabolic phenotypes, responses to perturbations as well as for the identification of differences and commonalities between individual organisms.

In this review, we will outline the -omics and systems biology contributions in the *Aspergillus* genus, and how they have made them one of the leading genera in omics-based research, if not *the* leading genus.

2. Systems Biology in Aspergilli

An important development for the understanding of biology on a systems level is the development in the field of the ”omics” technologies i.e. genomics, transcriptomics, proteomics and metabolomics. These technologies allow the acquisition of a snapshot of state of the system allowing the unbiased observation of system-wide effects induced by environmental and temporal changes.

2.1. Genomics

Genomics research in the genus of *Aspergillus* has received most attention since the start of the genomics era in filamentous fungi. Even though the Neurospora community published the fungal genome for *Neurospora crassa* in 2003[7], the Aspergillus field was quick to follow with the triple publications of *A. nidulans*[8], *A. oryzae*[9], and *A. fumigatus*[10] in a 2005 issue of Nature.

Since the publication of the first three *Aspergillus* genomes, genomes sequenced from the *Aspergillus* genus have been diverse and plentiful. First, the Broad Institute released the genome of *A. terreus* in 2006, followed by the sequencing of *A. niger* CBS 513.88[11], an important industrial work horse. A large number of single genomes have been published since then, such as *A. parasiticus*[12], *A. udagawae*[13], *A. flavus*[14], *A. calidoustus*[15], *A. luchuensis*[16], *A. bombycis*[17], or even isolates from the International Space Station[18] to name a few.

A part of the success of genomics research in *Aspergilli* is the community support as seen in the manual curation of the *A. nidulans* genome[19]. The activity of the community is also evident in the establishment of the active and curated genome repository, the Aspergillus genome database(AspGD)[20, 21], which has now been continued in FungiDB[22].

A large part of the genomics research in Aspergilli has been published in the form of comparative genomics studies, where new genomes are compared to previously published ones. One notable example is the first comparative study of the genome sequence of three strains of the same species, the wild type citric acid producer *A. niger* ATCC 1015, the industrial enzyme-producer *A. niger* CBS 513.88, and the NRRL3 laboratory strain[23]. In a similar study performed in 2008[24] the authors compared two isolates of *A. fumigatus*. From these studies it became particularly evident that there is a large amount of species diversity at the genomic level in Aspergilli. Comparative genomics has also been employed for analysis of diversity in published genomes for specific traits, e.g. intragenic tandem repeats[25] or carbohydrate-active enzymes[26], showing how re-analysis of genomes across specific sets can generate new insights.

Recently, Aspergillus comparative genomics has increased in scale. The most prominent example is the community-driven analysis of 19 *Aspergillus* genomes, 10 of them from new *de novo* genomes[27]. This type of analysis will only expand in the near future, as a part of the community is sequencing and annotating a member of all ¿350 species currently de- fined in the *Aspergillus genus*. This project has so far released genomes for more than 25 *Aspergillus* species, and have completed an additional 150 which are about to be published. The prospects of having access to the diversity of *Aspergilli* at the genome level are fascinating and will allow many future studies of fungal genomics at so far unseen levels. This has also led to proposals of similar studies for the *Penicillium* and *Trichoderma* genera. Thus, the extent and diversity of *Aspergillus* genomics has become leading in the area of fungal genomics, and is expected to remain so for the foreseeable future.

2.2. Transcriptomics

The availability of selected genomes of the Aspergillus genus allowed it to enter the transcriptomics era relatively early[28, 29]. With microarrays being available, the first comparisons of system-wide effects of experimental perturbations, in some cases called comparative transcriptomcs[29, 30, 31] was possible. Another application has been the early (2011) single-cell study of hyphal differentiation[32] in *A. niger*.

The Aspergillus area has also been relatively early adopters of RNA-seq, with the first study based on *A. oryzae* published in 2010[33], and currently about 30 studies published.

One specific and efficient application of transcriptomics data is the elucidation and study of metabolic pathways. Using comparative transcriptomics between two nutrient conditions allowed the putative identification of genes involved in the catabolism of galactaric acid by Kuivanen et al.[34] by screening for upregulated genes, or in another case to identify genes involved in itaconic acid production[35].

Another powerful area of application for transcriptomics based experiments has been the observation of global changes to environmental stresses like hypoxia[36, 37, 38], pH[39], and heat[10], shedding light on mechanisms allowing filamentous fungi to cope with these changes that might lead to insights for understanding fungal durability and towards targets for combating them[40]. In the case of hypoxia, *A. fumigatus* seems to possess a robust cellular response involving the upregulation of e.g. glycolysis, iron uptake and ergosterol biosynthesis and also led to the identification of a potential antifungal target in the Q10 biosynthesis pathway[41].

In summary, *Aspergillus* transcriptomics studies are abundant and have given insight on key traits in the fungal lifestyle and differentiation, to name just a few areas.

2.3. Proteomics

As transcriptomics analysis is frequently used as an approximation for proteomic changes it is not surprising that proteomics data has been used in similar ways as described for transcriptomics data above. An area of special interest in the proteomics of Aspergilli has been the set of secreted biomass degrading enzymes.

One example for the analysis of proteomics data for differences in the secretome between conditions is the study by Lu et al.[42]. The authors collected data for both the intra- and extracellular proteome under different conditions thereby proving and excellent insight conditional changes in the proteome of *A. niger*. A similar study comparing the secretome of *A. fumigatus* on three different carbon sources[43] additionally found many enzymes to be post-translationally modified by deamination. Comparing substrate dependent differences in membrane associated proteins has led to the identification of transporters for glucose[44], as well as to the first eukaryotic L-rhamnose transporter[45].

The combination of proteomics and transcriptomics have also been used as an important tool in understanding the development of several Aspergilli. As filamentous fungi propagate by forming conidia spores, processes governing the germination of spores are of vital importance. Using a combination of transcriptomic and proteomic data, Novodvorska et al.[46] have been able to show, that fungal spores are still metabolically active and switch from fermentative to respiratory growth upon triggering outgrowth of the spores. Similarly Anjo et al.[47] found that spores of *A. fumigatus* proceed from a metabolic active state via dormancy to an autophagic state under nutrient limitation.

Integration of multi-omics data sets has proven useful for the identification of genes contributing to complex biological traits like protein secretion. A notable example for the integration and transcriptomics data is the identification of leads for strain improvement by Jacobs et al.[48]. Using the combination of these technologies allowed the authors to identify metabolic engineering targets based on the correlation of changes in transcript with changes

in protein level. Those genes represent good candidates for genetic engineering as there is no obvious regulation on another level like post-translational regulation.

2.4. *Metabolomics*

Metabolomics on a systems level has been applied to analyze the underlying mechanism of productivity by comparing strains with varying levels of production. Metabolic flux analysis (MFA) is used for the investigation of intracellular carbon fluxes and has been applied to *A. niger* and *A. oryzae*. Comparing the flux distribution of *A. niger* overexpressing fructofuranosidase to the parent strain Driouch et al.[49] found a redistribution the metabolic flux to the Pentose Phosphate Pathway (PPP) leading to an increase in NADPH production. The same observation has been made by Lu et al.[50] in high glucoamylase producer of *A. niger* as well as by Pedersen et al.[51] in *A. oryzae* producing alpha-amylase. The observed modulation of the flux through the PPP therefore appears to be a general feature of Aspergilli expressing recombinant proteins.

Another area of research that has gained a lot of interest in Aspergilli is the biosynthesis of secondary metabolites. The diversity of secondary metabolites produced by different Aspergilli has been reviewed elsewhere[1] and the information about individual metabolites has been collected in the A2MDB database[52]. One of the challenges in this area is that most of the biosynthetic gene clusters are inactive under standard laboratory conditions. Targeted methods employing promoter exchange of individual genes[53] or heterologous expression[54] as well as untargeted approaches like epigenetic modifiers[55] have proven successful in identifying new compounds and the corresponding gene clusters.

2.5. *Modelling*

Mathematical modelling is at the core of systems biology in order to integrate information and quantitatively analyze phenotypes thereby allowing predictions about the behavior of the organism or elucidating mechanisms underlying experimental observations. The organ- ism which sparked the most interest in developing a model is *A. niger* and the unique citric acid accumulation phenotype. The metabolic network of *A. niger* has therefore been subject to several modelling efforts to elucidate key aspects of the high citric acid production. Stoichiometric modelling has been pioneered in filamentous fungi with the reconstruction of the central metabolism of *A. niger* [56] and *A. nidulans* [57] which paved the way for the development of genome-scale models. The first genome-scale model of *A. niger* has been developed by our group in 2008[58] and has recently been updated by Liu et al.[59]. Similarly genome-scale models are available for *A. terreus* [60], *A. oryzae* [61] and *A. nidulans* [62].

Besides these genome-scale stoichiometric models, kinetic models have been developed and analyzed for different aspects of Aspergillus physiology. Separate models have been developed for the catabolism of arabinose[63] and xylose[64] as well as for the citric acid production[65] in *A. niger* in order to identify key enzymatic steps as metabolic engineering targets.

3. Discussion

As reviewed above, the early adaptation of omics-methods *Aspergillus* research has brought significant contributions within a broad range of omics, showing how the Aspergilli are suitable models for study of fungal-specific, microbe-specific, and general eukaryotic traits. Seeing how rapid the development has been since the publication of the first genomes in 2005, it will be interesting to follow how the field develops. Based on the recent developments in the area, we will hazard some guesses.

One trend that we believe to see coming up, is how future research in Aspergilli will shift more towards the development and utilization of high-throughput technologies. One of the major hurdles for performing genetic research in Aspergilli in a systematic manner has been the low efficiency for targeted integration and gene knockout. Since the introduction of the CRISPR/Cas9 technology to Aspergilli[66] the efficiency of targeted genetic manipulation has been boosted significantly[34, 67, 68] enabling the assessment of a large number of those modifications in a high-throughput manner. Leveraging this gain in strain development will speed up the elucidation of pathways both in primary and secondary metabolism as well as enabling the development of genome-wide screens e.g. knockout libraries as has been performed in other model systems[69]. Similarly a catalytically inactive variant of Cas9 (dCas9) might be used for high-throughput elucidation of transcriptional regulation by performing knock-down or activation experiments of individual transcription factors.

Another development could be faster identification of genes contributing to a specific phenotype. One example of this trend is the elucidation of secondary metabolite biosynthesis. Today the identification of new secondary metabolites and the mapping to the corresponding gene-cluster is cumbersome. Several efforts have been made in the past in order to speed up the process and developing methods for scaling-up this process leading to a more rapid screening of whole libraries of gene clusters[54]. In combination with large-scale comparative genomics, as seen in *Penicillium* [70], this could accelerate the field.

One of the existing challenges for a true understanding of the system is the lack of information about the function of several genes[71]. At the same time, information about the subcellular localization of individual proteins is even scarcer than for functional annotation, but has been shown to even be important for understanding the cellular context of known functions[72].

A final trend concerns how the masses of new data will be treated and stored. As the field is moving towards larger scale experimental work as well as the acquisition of increasing amounts of data per experiment, new challenges with respect to storing, handling and integration of this data arise in order to enable a systems level understanding. Some of the challenges for future research will be the management and the infrastructure for handling and integrating omics-data and experimental data generated by high-throughput experiments. At the moment, since data storage and metadata is not standardized, a lot of omics-data becomes ”single-use”, meaning that it is only analyzed in the original publication, due to poor accessibility for other researchers. This is both a waste and slows down the progress of the field. One of the initiatives aiming at developing solutions for this problem is the EU project called ELIXIR which aims at developing a joint infrastructure for life science research.

While storage and sharing of this data is a challenge in itself, the integration and joint interpretation tops the challenges of data handling in complexity. As biological systems are inherently complex, a systems level understanding will only be achieved by integrating all

available data in a coherent framework. Fungal research is already moving in this direction with the integration of large datasets in FungiDB and its sophisticated methods for data queries, but sustained support and use of these functions will be required for reaching the full potential of genome-wide studies and engineering.

4. Citations to highlight

*Galagan 2005 [8]
Early comparative genomics study of 3 Aspergillus species demonstrating conservation of non-coding regulatory regions between those organisms

**De Vries 2017 [27]
Publication and comparative analysis of 10 new Aspergillus genomes and comprehensive review of research considering metabolism and genetics in this field

*De Bekker 2011 [32]
First study of single cell transcriptomics in Aspergilli demonstrating the heterogeneity of individual hyphae

*Lu 2010 [42]
Excellent proteomics study in Aspergillus niger, providing data for both the extracellular and intracellular proteome and their change to nutritional condition

**Nodvig 2015 [66]
First application of the CRISPR/Cas9 system in filamentous fungi, proving far superior gene efficiency compared to traditional methods.

*Clevenger 2017 [54]
Example of the development towards the high-throughput screening and identification of secondary metabolites as well as the clusters encoding the biosynthetic genes

5. Acknowledgement

The authors acknowledge funding from the Novo Nordisk Foundation, Grant NNF13OC0004831, Biotechnology-based Synthesis and Production.

References

1. Frisvad JC, Larsen TO: **Chemodiversity in the genus Aspergillus.** *Appl Microbiol Biotechnol* 2015, **99**:7859–7877.
2. Benoit I, Culleton H, Zhou M, DiFalco M, Aguilar-Osorio G, Battaglia E, Bouzid O, Brouwer CPJM, El-Bushari HBO, Coutinho PM, et al.: **Closely related fungi employ diverse enzymatic strategies to degrade plant biomass**. *Biotechnol Biofuels* 2015, **8**:107.
3. Dubey MK, Zehra A, Aamir M, Meena M, Ahirwal L, Singh S, Shukla S, Upadhyay RS, Bueno-Mari R, Bajpai VK: **Improvement Strategies, Cost Effective Production, and Potential Applications of Fungal Glucose Oxidase (GOD): Current Updates.** *Front Microbiol* 2017, **8**:1032.
4. Max B, Salgado JM, Rodríguez N, Cortés S, Converti A, Domínguez JM: **Biotechnological production of citric acid**. *Brazilian J Microbiol* 2010, **41**:862–875.
5. Sewalt V, Shanahan D, Gregg L, La Marta J, Carrillo R: **The Generally Recognized as Safe (GRAS) Process for Industrial Microbial Enzymes**. *Ind Biotechnol* 2016, **12**:295–302.
6. Paulussen C, Hallsworth JE, Álvarez-Pérez S, Nierman WC, Hamill PG, Blain D, Rediers H, Lievens B: **Ecology of aspergillosis: insights into the pathogenic potency of *Aspergillus fumigatus* and some other Aspergillus species.** *Microb Biotechnol* 2017, **10**:296–322.
7. Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D, FitzHugh W, Ma L-J, Smirnov S, Purcell S, et al.: **The genome sequence of the filamentous fungus Neurospora crassa**. *Nature* 2003, **422**:859–868.
8. Galagan JE, Calvo SE, Cuomo C, Ma L-J, Wortman JR, Batzoglou S, Lee S-I, Baştürkmen M, Spevak CC, Clutterbuck J, et al.: **Sequencing of *Aspergillus nidulans* and comparative analysis with A. fumigatus and A. oryzae**. *Nature* 2005, **438**:1105–1115.
9. Machida M, Asai K, Sano M, Tanaka T, Kumagai T, Terai G, Kusumoto K-I, Arima T, Akita O, Kashiwagi Y, et al.: **Genome sequencing and analysis of *Aspergillus oryzae***. *Nature* 2005, **438**:1157–1161.
10. Nierman WC, Pain A, Anderson MJ, Wortman JR, Kim HS, Arroyo J, Berriman M, Abe K, Archer DB, Bermejo C, et al.: **Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus***. *Nature* 2005, **438**:1151–1156.
11. Pel HJ, de Winde JH, Archer DB, Dyer PS, Hofmann G, Schaap PJ, Turner G, de Vries RP, Albang R, Albermann K, et al.: **Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88**. *Nat Biotechnol* 2007, **25**:221–231.
12. Linz JE, Wee J, Roze L V: **Aspergillus parasiticus SU-1 Genome Sequence, Predicted Chromosome Structure, and Comparative Gene Expression under Aflatoxin-Inducing Conditions: Evidence that Differential Expression Contributes to Species Phenotype.** *Eukaryot Cell* 2014, **13**:1113–1123.
13. Kusuya Y, Takahashi-Nakaguchi A, Takahashi H, Yaguchi T: **Draft Genome Sequence of the Pathogenic Filamentous Fungus *Aspergillus udagawae* Strain IFM 46973 T**. *Genome Announc* 2015, **3**:e00834-15.
14. Nierman WC, Yu J, Fedorova-Abrams ND, Losada L, Cleveland TE, Bhatnagar D, Bennett JW, Dean R, Payne GA: **Genome Sequence of *Aspergillus flavus* NRRL 3357, a Strain That Causes Aflatoxin Contamination of Food and Feed**. *Genome Announc* 2015, **3**:e00168--15.
15. Horn F, Linde J, Mattern DJ, Walther G, Guthke R, Scherlach K, Martin K, Brakhage AA, Petzke L, Valiante V: **Draft Genome Sequences of Fungus *Aspergillus calidoustus*.** *Genome Announc* 2016, **4**:e00102--16.
16. Yamada O, Machida M, Hosoyama A, Goto M, Takahashi T, Futagami T, Yamagata Y, Takeuchi M, Kobayashi T, Koike H, et al.: **Genome sequence of *Aspergillus luchuensis* NBRC 4314**. *DNA Res* 2016, **23**:507–515.
17. Moore GG, Mack BM, Beltz SB, Gilbert MK: **Draft Genome Sequence of an Aflatoxigenic Aspergillus Species, *A. bombycis***. *Genome Biol Evol* 2016, **8**:3297–3300.
18. Singh NK, Blachowicz A, Checinska A, Wang C, Venkateswaran K: **Draft Genome Sequences of Two *Aspergillus fumigatus* Strains, Isolated from the International Space Station**. *Genome Announc* 2016, **4**:e00553-16.
19. Wortman JR, Gilsenan JM, Joardar V, Deegan J, Clutterbuck J, Andersen MR, Archer D, Bencina M, Braus G, Coutinho P, et al.: **The 2008 update of the *Aspergillus nidulans* genome annotation: a community effort.** *Fungal Genet Biol* 2009, **46 Suppl 1**:S2--13.
20. Arnaud MB, Cerqueira GC, Inglis DO, Skrzypek MS, Binkley J, Chibucos MC, Crabtree J, Howarth C, Orvis J, Shah P, et al.: **The Aspergillus Genome Database (AspGD): recent developments in comprehensive multispecies curation, comparative genomics and community resources.** *Nucleic Acids Res* 2012, **40**:D653----D659.
21. Cerqueira GC, Arnaud MB, Inglis DO, Skrzypek MS, Binkley G, Simison M, Miyasato SR, Binkley J, Orvis J, Shah P, et al.: **The Aspergillus Genome Database: Multispecies curation and incorporation of RNA-Seq data to improve structural gene**

annotations. *Nucleic Acids Res* 2014, **42**:705–710.
22. Stajich JE, Harris T, Brunk BP, Brestelli J, Fischer S, Harb OS, Kissinger JC, Li W, Nayak V, Pinney DF, et al.: **FungiDB: An integrated functional genomics database for fungi**. *Nucleic Acids Res* 2012, **40**:675–681.
23. Andersen MR, Salazar MP, Schaap PJ, van de Vondervoort PJI, Culley D, Thykaer J, Frisvad JC, Nielsen KF, Albang R, Albermann K, et al.: **Comparative genomics of citric-acid-producing *Aspergillus niger* ATCC 1015 versus enzyme-producing CBS 513.88.** *Genome Res* 2011, **21**:885–97.
24. Fedorova ND, Khaldi N, Joardar VS, Maiti R, Amedeo P, Anderson MJ, Crabtree J, Silva JC, Badger JH, Albarraq A, et al.: **Genomic islands in the pathogenic filamentous fungus *Aspergillus fumigatus*.** *PLoS Genet* 2008, **4**:e1000046.
25. Gibbons JG, Rokas A: **Comparative and functional characterization of intragenic tandem repeats in 10 Aspergillus genomes**. *Mol Biol Evol* 2009, **26**:591–602.
26. Coutinho PM, Andersen MR, Kolenova K, VanKuyk PA, Benoit I, Gruben BS, Trejo-Aguilar B, Visser H, van Solingen P, Pakula T, et al.: **Post-genomic insights into the plant polysaccharide degradation potential of *Aspergillus nidulans* and comparison to *Aspergillus niger* and *Aspergillus oryzae*.** *Fungal Genet Biol* 2009, **46 Suppl 1**:S161----S169.
27. de Vries RP, Riley R, Wiebenga A, Aguilar-Osorio G, Amillis S, Uchima CA, Anderluh G, Asadollahi M, Askin M, Barry K, et al.: **Comparative genomics reveals high biological diversity and specific adaptations in the industrially and medically important fungal genus Aspergillus.** *Genome Biol* 2017, **18**:28.
28. da Silva Ferreira ME, Malavazi I, Savoldi M, Brakhage AA, Goldman MHS, Kim HS, Nierman WC, Goldman GH: **Transcriptome analysis of *Aspergillus fumigatus* exposed to voriconazole.** *Curr Genet* 2006, **50**:32–44.
29. Andersen MR, Vongsangnak W, Panagiotou G, Salazar MP, Lehmann L, Nielsen J: **A trispecies Aspergillus microarray: comparative transcriptomics of three Aspergillus species.** *Proc Natl Acad Sci U S A* 2008, **105**:4387–4392.
30. Salazar M, Vongsangnak W, Panagiotou G, Andersen M, Nielsen J: **Uncovering transcriptional regulation of glycerol metabolism in Aspergilli through genome-wide gene expression data analysis.** *Mol Genet Genomics* 2009, **282**:571–586.
31. Terabayashi Y, Shimizu M, Kitazume T, Masuo S, Fujii T, Takaya N: **Conserved and specific responses to hypoxia in *Aspergillus oryzae* and *Aspergillus nidulans* determined by comparative transcriptomics**. *Appl Microbiol Biotechnol* 2012, **93**:305–317.
32. de Bekker C, Bruning O, Jonker MJ, Breit TM, Wösten HA: **Single cell transcriptomics of neighboring hyphae of *Aspergillus niger***. *Genome Biol* 2011, **12**:R71.
33. Wang B, Guo G, Wang C, Lin Y, Wang X, Zhao M, Guo Y, He M, Zhang Y, Pan L: **Survey of the transcriptome of *Aspergillus oryzae* via massively parallel mRNA sequencing**. *Nucleic Acids Res* 2010, **38**:5075–5087.
34. Kuivanen J, Wang Y-MJ, Richard P: **Engineering *Aspergillus niger* for galactaric acid production: elimination of galactaric acid catabolism by using RNA sequencing and CRISPR/Cas9**. *Microb Cell Fact* 2016, **15**:210.
35. Li A, van Luijk N, ter Beek M, Caspers M, Punt P, van der Werf M: **A clone-based transcriptomics approach for the identification of genes relevant for itaconic acid production in Aspergillus**. *Fungal Genet Biol* 2011, **48**:602–611.
36. Barker BM, Kroll K, Vödisch M, Mazurie A, Kniemeyer O, Cramer RA: **Transcriptomic and proteomic analyses of the *Aspergillus fumigatus* hypoxia response using an oxygen-controlled fermenter**. *BMC Genomics* 2012, **13**:62.
37. Losada L, Barker BM, Pakala S, Pakala S, Joardar V, Zafar N, Mounaud S, Fedorova N, Nierman WC, Cramer RA: **Large-Scale Transcriptional Response to Hypoxia in *Aspergillus fumigatus* Observed Using RNAseq Identifies a Novel Hypoxia Regulated ncRNA**. *Mycopathologia* 2014, **178**:331–339.
38. Kroll K, Pahtz V, Hillmann F, Vaknin Y, Schmidt-Heck W, Roth M, Jacobsen ID, Osherov N, Brakhage AA, Kniemeyer O: **Identification of Hypoxia-Inducible Target Genes of *Aspergillus fumigatus* by Transcriptome Analysis Reveals Cellular Respiration as an Important Contributor to Hypoxic Survival**. *Eukaryot Cell* 2014, **13**:1241–1253.
39. Andersen MR, Lehmann L, Nielsen J: **Systemic analysis of the response of *Aspergillus niger* to ambient pH.** *Genome Biol* 2009, **10**:R47.
40. Brown NA, Goldman GH: **The contribution of *Aspergillus fumigatus* stress responses to virulence and antifungal resistance**. *J Microbiol* 2016, **54**:243–253.
41. Kroll K, Shekhova E, Mattern DJ, Thywissen A, Jacobsen ID, Strassburger M, Heinekamp T, Shelest E, Brakhage AA, Kniemeyer O: **The hypoxia-induced dehydrogenase HorA is required for coenzyme Q10 biosynthesis, azole sensitivity and virulence of *Aspergillus fumigatus*.** *Mol Microbiol* 2016, **101**:92–108.
42. Lu X, Sun J, Nimtz M, Wissing J, Zeng A-P, Rinas U: **The intra- and extracellular proteome of *Aspergillus niger* growing on defined medium with xylose or maltose as carbon substrate.** *Microb Cell Fact* 2010, **9**:23.
43. Adav SS, Ravindran A, Sze SK: **Quantitative proteomic study of *Aspergillus Fumigatus* secretome revealed deamidation of secretory enzymes**. *J Proteomics* 2015, **119**:154–168.
44. Sloothaak J, Odoni DI, de Graaff LH, Martins Dos Santos VAP, Schaap PJ, Tamayo-Ramos JA: ***Aspergillus niger* membrane-associated proteome analysis for the identification of glucose transporters.** *Biotechnol Biofuels* 2015, **8**:150.
45. Sloothaak J, Odoni DI, Martins Dos Santos VAP, Schaap PJ, Tamayo-Ramos JA: **Identification of a Novel L-rhamnose Uptake**

Transporter in the Filamentous Fungus *Aspergillus niger*. *PLoS Genet* 2016, **12**:e1006468.
46. Novodvorska M, Stratford M, Blythe MJ, Wilson R, Beniston RG, Archer DB: **Metabolic activity in dormant conidia of *Aspergillus niger* and developmental changes during conidial outgrowth.** *Fungal Genet Biol* 2016, **94**:23–31.
47. Anjo SI, Figueiredo F, Fernandes R, Manadas B, Oliveira M: **A proteomic and ultrastructural characterization of *Aspergillus fumigatus*' conidia adaptation at different culture ages.** *J Proteomics* 2017, **161**:47–56.
48. Jacobs DI, Olsthoorn MMA, Maillet I, Akeroyd M, Breestraat S, Donkers S, van der Hoeven RAM, van den Hondel CAMJJ, Kooistra R, Lapointe T, et al.: **Effective lead selection for improved protein production in *Aspergillus niger* based on integrated genomics**. *Fungal Genet Biol* 2009, **46**:S141–S152.
49. Driouch H, Melzer G, Wittmann C: **Integration of in vivo and in silico metabolic fluxes for improvement of recombinant protein production.** *Metab Eng* 2012, **14**:47–58.
50. Lu H, Liu X, Huang M, Xia J, Chu J, Zhuang Y, Zhang S, Noorman H: **Integrated isotope-assisted metabolomics and (13)C metabolic flux analysis reveals metabolic flux redistribution for high glucoamylase production by *Aspergillus niger*.** *Microb Cell Fact* 2015, **14**:147.
51. Pedersen, Carlsen, Nielsen: **Identification of enzymes and quantification of metabolic fluxes in the wild type and in a recombinant *Aspergillus oryzae* strain**. *Appl Environ Microbiol* 1999, **65**:11–19.
52. Vadlapudi V, Borah N, Yellusani KR, Gade S, Reddy P, Rajamanikyam M, Vempati LNS, Gubbala SP, Chopra P, Upadhyayula SM, et al.: **Aspergillus Secondary Metabolite Database, a resource to understand the Secondary metabolome of Aspergillus genus.** *Sci Rep* 2017, **7**:7325.
53. Yeh H-H, Ahuja M, Chiang Y-M, Oakley CE, Moore S, Yoon O, Hajovsky H, Bok J-W, Keller NP, Wang CCC, et al.: **Resistance Gene-Guided Genome Mining: Serial Promoter Exchanges in *Aspergillus nidulans* Reveal the Biosynthetic Pathway for Fellutamide B, a Proteasome Inhibitor.** *ACS Chem Biol* 2016, **11**:2275–2284.
54. Clevenger KD, Bok JW, Ye R, Miley GP, Verdan MH, Velk T, Chen C, Yang K, Robey MT, Gao P, et al.: **A scalable platform to identify fungal secondary metabolites and their gene clusters**. *Nat Chem Biol* 2017, **13**:895–901.
55. Albright JC, Henke MT, Soukup AA, McClure RA, Thomson RJ, Keller NP, Kelleher NL: **Large-scale metabolomics reveals a complex response of *Aspergillus nidulans* to epigenetic perturbation.** *ACS Chem Biol* 2015, **10**:1535–1541.
56. David H, Akesson M, Nielsen J: **Reconstruction of the central carbon metabolism of *Aspergillus niger*.** *Eur J Biochem* 2003, **270**:4243–53.
57. David H, Hofmann G, Oliveira AP, Jarmer H, Nielsen J: **Metabolic network driven analysis of genome-wide transcription data from *Aspergillus nidulans*.** *Genome Biol* 2006, **7**:R108.
58. Andersen MR, Nielsen ML, Nielsen J: **Metabolic model integration of the bibliome, genome, metabolome and reactome of *Aspergillus niger*.** *Mol Syst Biol* 2008, **4**:178.
59. Lu H, Cao W, Ouyang L, Xia J, Huang M, Chu J, Zhuang Y, Zhang S, Noorman H: **Comprehensive reconstruction and in silico analysis of Aspergillus niger genome-scale metabolic network model that accounts for 1210 ORFs.** *Biotechnol Bioeng* 2017, **114**:685–695.
60. Liu J, Gao Q, Xu N, Liu L: **Genome-scale reconstruction and in silico analysis of *Aspergillus terreus* metabolism.** *Mol Biosyst* 2013, **9**:1939–1948.
61. Vongsangnak W, Olsen P, Hansen K, Krogsgaard S, Nielsen J: **Improved annotation through genome-scale metabolic modeling of *Aspergillus oryzae*.** *BMC Genomics* 2008, **9**:245.
62. David H, Ozçelik IS, Hofmann G, Nielsen J: **Analysis of *Aspergillus nidulans* metabolism at the genome-scale.** *BMC Genomics* 2008, **9**:163.
63. Prathumpai W, Gabelgaard JB, Wanchanthuek P, Van de Vondervoort PJI, de Groot MJL, McIntyre M, Nielsen J: **Metabolic Control Analysis of Xylose Catabolism in Aspergillus**. *Biotechnol Prog* 2008, **19**:1136–1141.
64. deGroot MJL, Prathumpai W, Visser J, Ruijter GJG: **Metabolic Control Analysis of *Aspergillus niger* L-Arabinose Catabolism**. *Biotechnol Prog* 2005, **21**:1610–1616.
65. Alvarez-Vasquez F, González-Alcón C, Torres N V: **Metabolism of citric acid production by *Aspergillus niger*: model definition, steady-state analysis and constrained optimization of citric acid production rate.** *Biotechnol Bioeng* 2000, **70**:82–108.
66. Nødvig CS, Nielsen JB, Kogle ME, Mortensen UH: **A CRISPR-Cas9 System for Genetic Engineering of Filamentous Fungi**. *PLoS One* 2015, **10**:e0133085.
67. Fuller KK, Chen S, Loros JJ, Dunlap JC: **Development of the CRISPR/Cas9 System for Targeted Gene Disruption in *Aspergillus fumigatus***. *Eukaryot Cell* 2015, **14**:1073–1080.
68. Katayama T, Tanaka Y, Okabe T, Nakamura H, Fujii W, Kitamoto K, Maruyama J-I: **Development of a genome editing technique using the CRISPR/Cas9 system in the industrial filamentous fungus *Aspergillus oryzae*.** *Biotechnol Lett* 2016, **38**:637–42.
69. Nislow C, Wong LH, Lee AH-Y, Giaever G: **Functional Genomics Using the *Saccharomyces cerevisiae* Yeast Deletion**

Collections. *Cold Spring Harb Protoc* 2016, **2016**:pdb.top080945.

70. Nielsen JC, Grijseels S, Prigent S, Ji B, Dainat J, Nielsen KF, Frisvad JC, Workman M, Nielsen J: **Global analysis of biosynthetic gene clusters reveals vast potential of secondary metabolite production in Penicillium species**. *Nat Microbiol* 2017, **2**:17044.
71. Andersen MR: **Elucidation of primary metabolic pathways in Aspergillus species: Orphaned research in characterizing orphan genes**. *Brief Funct Genomics* 2014, **13**:451–455.
72. Aguilar-Osorio G, VanKuyk PA, Seiboth B, Blom D, Solomon PS, Vinck A, Kindt F, Wosten HAB, de Vries RP: **Spatial and Developmental Differentiation of Mannitol Dehydrogenase and Mannitol-1-Phosphate Dehydrogenase in *Aspergillus niger***. *Eukaryot Cell* 2010, **9**:1398–1402.

- Aspergillus has been a model genus for fungal omics research, with a diverse set of species in the genus studied

- Multi-omics research has allowed systems level insight into important traits like high protein secretion and stress responses

- CRISPR/Cas9 has increased the speed of genetic research

- Increase in data will lead to new challenges in data handling and interpretation