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A public resource for metabolic pathway mapping of *Aspergillus fumigatus* Af293

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Our understanding of the human pathogenic fungus *Aspergillus fumigatus* has recently benefitted from much work at the genomics level, including genome sequencing and comparative genome analyses. The next stage in this process is to develop a higher-level appreciation of the organism's biology and to this end the Pathway Tools software has been used to map the metabolic pathways of *A. fumigatus* Af293. The resulting web-based resource shows 242 pathways which can be viewed at a variety of resolutions. Some pathways have been manually curated (e.g., ergosterol biosynthesis, 4-hydroxymandelate degradation, fatty acid β-oxidation, fatty acid ω-oxidation, the glyoxylate cycle, palmitate biosynthesis, pyridoxal 5′-phosphate salvage, sphingolipid metabolism, ubiquinone biosynthesis and very long chain fatty acid biosynthesis) while others can be used as a starting point for more detailed research. The pathways can be viewed via the Scientific Information:Genomes section of the Aspergillus website (www.aspergillus.org.uk).

Keywords *Aspergillus fumigates*, pathway, ergosterol, metabolism

Introduction

The filamentous fungus *Aspergillus fumigatus* is an important invasive pathogen of immunocompromised humans and a producer of many allergen proteins. While the incidence of invasive aspergillosis (IA) cases is not high (~5 cases per year in a group of surveyed hospitals [1]; IA in ~2% of haematopoetic stem cell transplants [2]), crude mortality rates of ~50–80% have been reported for patients with aspergillosis [2]. As a consequence, considerable effort is being expended in the improvement of our understanding of *A. fumigatus* biology, as well as in the identification of anti-fungal drugs. Much has been achieved at the genome level, with the completion of the *A. fumigatus* Af293 genome sequence [3], the publication of comparative genome analyses [4,5] and the provision of the publicly available genomics resources CADRE [6], Ensembl fungi [7] and AspGD [8]. The next step is to develop a better understanding of *A. fumigatus* at a higher organizational level, for example, by focusing on metabolomics. An extensive metabolic model for the related organism *A. niger* was recently developed by Andersen *et al*. [9]. We present here a publicly available resource for the visualization of metabolic pathways in *A. fumigatus* Af293.

Methods

The Pathway Tools v13.5 software [10,11] was obtained from SRI International, USA. The annotated *A. fumigatus* Af293 genome was downloaded from Ensembl fungi (ftp://ftp.ensemblgenomes.org/pub/fungi/release-3/genbank/aspergillus_fumigatus) and modified using bioperl modules firstly to render the intron/exon structure lines into GenBank format, and secondly to move enzyme annotations and EC numbers into the CDS field. Pathway building was carried out according to the handbook accompanying the software; the standard operating procedure from PATRIC was also used (http://patric.vbi.vt.edu/about/SOP/PDC_v1.2.pdf).

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Automatic identification and assignment of enzymes using the PathoLogic tool resulted in 244 pathways. A pathway hole report was then generated, identifying 276 'holes', i.e., reactions to which no enzyme had been assigned. These holes were manually inspected to identify and assign a gene where possible. In many cases the following strategies were used: (i) searches of GenBank using enzyme names or EC numbers; (ii) Blast searches of *A. fumigatus* sequences with annotated sequences from other organisms; (iii) Pubmed searches using enzyme names; (iv) Comparison with *Saccharomyces cerevisiae* pathways (http://pathway.yeastgenome.org/); (v) Comparison with the pathways available on MetaCyc (http://metacyc.org/; [12]). In some cases this optimization process led to the deletion of reactions or pathways if it seemed highly probable that these were absent from *A. fumigatus*. A number of pathways were examined in detail and all their component reactions checked.

Results

Automated analysis of the annotated *A. fumigatus* Af293 genome by Pathway Tools, followed by manual inspection of holes and certain key pathways resulted in a pathway database of 242 pathways and 1576 reactions (Table 1). Nine pathways were annotated in detail (annotation level 1 in Table 1), including examination of literature where appropriate, with attention being paid to ergosterol biosynthesis, 4-hydroxymandelate degradation, fatty acid β-oxidation, fatty acid ω-oxidation, the glyoxylate cycle, palmitate biosynthesis, pyridoxal 5′-phosphate salvage, sphingolipid metabolism, ubiquinone biosynthesis and very long chain fatty acid biosynthesis. The ergosterol biosynthesis pathway, 'alternative ergosterol biosynthesis pathway (*A. fumigatus*)', was of particular interest as it is the site of action of the antifungal azoles, which inhibit lanosterol demethylase (cyp51/ERG11), and also of the allylamines (such as terbinafine) which inhibit a preceding enzyme, squalene epoxidase (ERG7).

The pathway database can be accessed via the Scientific Information;Genomes section of the *Aspergillus* website (www.aspergillus.org.uk); pathways may be viewed and downloaded with different levels of detail and it is possible to examine annotations and the level of supporting evidence. PDF views of a number of key pathways will be made available at the *Aspergillus* website and it is anticipated that the 'knowledgebase' files will be made available for download so that local copies can be established by any laboratory running the Pathway Tools software.

Table 1 *Aspergillus fumigatus* Af293 pathways.

Pathway name	No. Steps[1]	Level[2]
2-amino-3-carboxymuconate semialdehyde degradation to glutaryl-coa	4 (1)	4
2-ketoglutarate dehydrogenase complex	3	3
2-methylcitrate cycle I	5 (2)	4
3-dehydroquinate biosynthesis I	2	3
4-aminobutyrate degradation I	3	3
4-hydroxybenzoate biosynthesis I (eukaryotes)	5 (1)	4
4-hydroxymandelate degradation[3]	**5**	**1**
5-aminoimidazole ribonucleotide biosynthesis I	5	3
6-hydroxymethyl-dihydropterin diphosphate biosynthesis	5 (3)	4
Acetate conversion to acetyl-coa	1	3
Acetoin biosynthesis I	3 (1)	4
Acetyl-coa biosynthesis (from citrate)	1	3
Acetyl-coa biosynthesis (from pyruvate)	3	3
Acyl-coa hydrolysis	1	3
Aerobic respiration—electron donor II	4	3
Aerobic respiration—electron donor III	4 (2)	4
Allantoin degradation to ureidoglycolate I (urea producing)	2	3
Alternative ergosterol biosynthesis pathway (*A. Fumigatus*)[4]	**17**	**1**
Arginine biosynthesis II (acetyl cycle)	9	3
Arginine degradation I (arginase pathway)	4 (3)	3
Arginine degradation VI (arginase 2 pathway)	4 (3)	3
Arginine degradation VII (arginase 3 pathway)	2	3
Arginine degradation X (arginine monooxygenase pathway)	3 (1)	4
Arsenate detoxification II	1	3
Asparagine biosynthesis I	1	3
Asparagine degradation I	1	3
Aspartate biosynthesis	1	3
Aspartate degradation II	2	3
Beta-alanine biosynthesis V	2 (1)	4
Betacyanin biosynthesis	7 (1)	4
Betanidin degradation	1	3
Betaxanthin biosynthesis	3 (2)	3
Betaxanthin biosynthesis (via dopamine)	2 (1)	3
Branched-chain alpha-keto acid dehydrogenase complex	3	2
Butanediol biosynthesis	1	3
Butanediol degradation	1	3
C30 botryococcene biosynthesis	3	2
Calcium transport I	2	3
Cardiolipin biosynthesis II	3 (2)	2
CDP-diacylglycerol biosynthesis I	4	2
Choline biosynthesis III	3	3
Choline degradation I	2	3
Choline degradation II	2	3
Chorismate biosynthesis I	7	2
Citrulline degradation	2 (1)	4
Coenzyme A biosynthesis	5	2
Copper transport II	1	3
Cyanate degradation	3	3
Cyclopropane fatty acid (CFA) biosynthesis	1	3
Degradation of purine ribonucleosides	6 (3)	4
DIMBOA-glucoside degradation	1	3
Di-trans,poly-cis-undecaprenyl diphosphate biosynthesis	8	3

(Continued)

Table 1 *(Continued).*

Pathway name	No. Steps[1]	Level[2]
D-mannose degradation	1	3
Dolichyl-diphosphooligosaccharide biosynthesis	16	2
Dtdp-L-rhamnose biosynthesis I	4 (1)	4
Epoxysqualene biosynthesis	3	3
Fatty acid activation	1	3
Fatty acid beta-oxidation I[5]	**7**	**1**
Fatty acid beta-oxidation II (core pathway)[5]	**5**	**1**
Fatty acid biosynthesis initiation II	2	3
Fatty acid omega-oxidation[6]	**6**	**1**
Flavin biosynthesis III (eukaryotes)	9 (8)	2
Folate polyglutamylation I	5	3
Folate polyglutamylation II	2 (1)	4
Folate transformations	12 (10)	2
Formaldehyde assimilation III (dihydroxyacetone cycle)	12	
Formate oxidation to CO2	1	3
Formate to nitrate electron transfer	2	3
Formylthf biosynthesis I	11	2
Formylthf biosynthesis II	12 (11)	2
Gamma-linolenate biosynthesis II (animals)	2 (1)	4
GDP-glucose biosynthesis	4 (2)	4
GDP-mannose biosynthesis	4	3
Geranyldiphosphate biosynthesis	1	3
Geranylgeranyldiphosphate biosynthesis	1	3
Gluconeogenesis I	12	
Glucose and glucose-1-phosphate degradation	4 (2)	4
Glutamate biosynthesis I	1	3
Glutamate biosynthesis III	1	3
Glutamate biosynthesis IV	1	3
Glutamate degradation I	1	3
Glutamate dependent acid resistance	1	3
Glutamine biosynthesis I	1	3
Glutamine degradation II	1	3
Glutaryl-coa degradation	5	2
Glutathione biosynthesis	2	3
Glutathione redox reactions I	3	2
Glutathione redox reactions II	2 (1)	3
Glutathione-mediated detoxification	1	2
Glycerol degradation I	3	3
Glycerol degradation IV	2	3
Glycerol-3-phosphate shuttle	2	3
Glycine betaine biosynthesis I (Gram-negative bacteria)	2	3
Glycine betaine biosynthesis II (Gram-positive bacteria)	2	3
Glycine biosynthesis I	1	3
Glycine biosynthesis III	1	3
Glycine biosynthesis IV	1	3
Glycine cleavage complex	3	3
Glycogen biosynthesis II (from UDP-D-Glucose)	5	3
Glycogen degradation I	7	3
Glycogen degradation II	5	3
Glycolysis II	10	3
Glyoxylate cycle	**6**	**1**
Heme biosynthesis from uroporphyrinogen-III I	4	3
Heptaprenyl diphosphate biosynthesis	4 (3)	2
Histidine biosynthesis	10	2
Homocysteine and cysteine interconversion	4 (3)	4
Homocysteine biosynthesis	2	3
Homogalacturonan degradation	2	3

(Continued)

Table 1 *(Continued).*

Pathway name	No. Steps[1]	Level[2]
Homoserine biosynthesis	3	3
Hyperforin biosynthesis	4 (2)	4
IAA biosynthesis V	1	3
Inosine-5′-phosphate biosynthesis II	5	3
Isoleucine biosynthesis I (from threonine)	5	3
Isoleucine degradation I	6	2
Isoleucine degradation II	3	2
Itaconate biosynthesis	4 (3)	4
Itaconate degradation	3 (1)	4
Lactose degradation III	1	3
Lanosterol biosynthesis	1	3
L-arabinose degradation II	3 (1)	4
L-cysteine degradation I	3 (2)	3
L-cysteine degradation II	1	3
Leucine biosynthesis	6 (5)	3
Leucine degradation I	7	2
Leucine degradation III	3	3
Lysine biosynthesis IV	8	2
Mannitol degradation I	1	3
Melibiose degradation	1	3
Methionine degradation I (to homocysteine)	3	3
Methionine degradation III	3 (2)	2
Methyl parathion degradation	1	3
Methylglyoxal degradation I	3	3
Methylglyoxal degradation V	3 (2)	4
Methylsalicylate degradation	2	3
Mevalonate pathway I	7	3
Mixed acid fermentation	11	4
Myo-inositol biosynthesis	2	3
N-acetylglucosamine degradation	2	3
N-acetylneuraminate and N-acetylmannosamine degradation	3 (1)	4
NAD biosynthesis from 2-amino-3-carboxymuconate semialdehyde	4 (3)	3
NAD biosynthesis I (from aspartate)	6 (3)	4
NAD salvage pathway I	8 (6)	2
NAD/NADH phosphorylation and dephosphorylation	6 (4)	4
Nitrate reduction III (dissimilatory)	2 (1)	4
Nitrate reduction V (assimilatory)	5 (4)	3
Oleate beta-oxidation	3	2
Oleate biosynthesis II (animals)	2 (1)	4
Ornithine biosynthesis	5	3
Ornithine degradation (proline biosynthesis)	1	3
Oxidative ethanol degradation I	3	3
Oxidative ethanol degradation III	3	3
Palmitate biosynthesis I (animals)	**29 (27)**	**1**
Pantothenate biosynthesis I	3	3
Paraoxon degradation	1	3
Pentose phosphate pathway (non-oxidative branch)	5	3
Pentose phosphate pathway (oxidative branch)	3	3
Pentose phosphate pathway (partial)	3	3
Phenol degradation I (aerobic)	1	3
Phenylalanine biosynthesis I	3	3
Phenylalanine degradation III	4 (2)	4
Phenylethanol biosynthesis	3 (2)	2
Phenylethylamine degradation	2 (1)	4
Phosphatidylcholine biosynthesis I	3	3
Phosphatidylcholine biosynthesis II	5	3
Phosphatidylcholine biosynthesis III	5 (3)	4

(Continued)

Table 1 *(Continued).*

Pathway name	No. Steps[1]	Level[2]
Phosphatidylcholine biosynthesis IV	5 (3)	4
Phosphatidylethanolamine biosynthesis I	2	3
Phosphatidylethanolamine biosynthesis II	3	3
Phospholipases	5	2
Proline biosynthesis I	4 (3)	3
Proline biosynthesis II (from arginine)	6 (3)	4
Proline degradation I	2	3
Proline degradation II	2	3
PRPP biosynthesis I	1	3
Purine deoxyribonucleosides degradation	7 (1)	4
Putrescine biosynthesis III	1	3
Pyridoxal 5′-phosphate biosynthesis	8 (2)	4
Pyridoxal 5′-phosphate salvage pathway	**5**	**1**
Pyrimidine deoxyribonucleosides degradation	6 (2)	4
Pyrimidine deoxyribonucleotides de novo biosynthesis	11 (8)	4
Pyrimidine ribonucleotides interconversion	4	2
Pyruvate fermentation to ethanol II	2	3
Pyruvate fermentation to lactate	1	3
Removal of superoxide radicals	2	3
Respiration (anaerobic)	7	2
S-adenosyl-L-methionine cycle II	4 (3)	4
S-adenosylmethionine biosynthesis	1	3
Salicylate degradation I	1	3
Salvage pathways of adenine, hypoxanthine, and their nucleosides	12 (3)	4
Salvage pathways of guanine, xanthine, and their nucleosides	7 (4)	2
Salvage pathways of pyrimidine ribonucleotides	8 (7)	2
Seed germination protein turnover	1	3
Selenocysteine biosynthesis II (archaea and eukaryotes)	4 (1)	4
Serine biosynthesis	3	3
Siroheme biosynthesis	4	2
Sorbitol biosynthesis II	3	3
Sorbitol degradation I	1	3
Spermidine biosynthesis	2	3
Sphingolipid metabolism[7]	**13**	**1**
Sphingomyelin metabolism	1	2
Sphingosine and sphingosine-1-phosphate metabolism	5 (4)	2
Sulfate activation for sulfonation	2	3
Sulfite oxidation IV	1	3
Taurine degradation IV	1	3
TCA cycle variation III (eukaryotic)	9	2
Tetrapyrrole biosynthesis II	4	3
Thioredoxin pathway	2 (1)	4
Thiosulfate disproportionation III (rhodanese)	1	3
Threonine biosynthesis from homoserine	2	3
Trans, trans-farnesyl diphosphate biosynthesis	3	3
Trehalose biosynthesis I	2	3
Trehalose degradation I (low osmolarity)	2	3
Trehalose degradation II (trehalase)	2	3
Triacylglycerol biosynthesis	5	3
Triacylglycerol degradation	4 (1)	4
Trna charging pathway	20	3
Tryptophan biosynthesis	6	2
Tryptophan degradation I (via anthranilate)	3	3
Tryptophan degradation to 2-amino-3-carboxymuconate semialdehyde	5	3
Tryptophan degradation X (mammalian, via tryptamine)	4	3
Tyrosine biosynthesis I	3	3
Tyrosine degradation I	5	2
Tyrosine degradation III	4 (2)	4
Ubiquinone-10 biosynthesis (eukaryotic)	**9 (6)**	**1**
Ubiquinone-6 biosynthesis (eukaryotic)	**9 (6)**	**1**
Ubiquinone-7 biosynthesis (eukaryotic)	**9 (6)**	**1**
Ubiquinone-8 biosynthesis (eukaryotic)	**9 (6)**	**1**
Ubiquinone-9 biosynthesis (eukaryotic)	**9 (6)**	**1**
UDP-N-acetyl-D-glucosamine biosynthesis II	4	3
Urate degradation to allantoin	3	2
Urea degradation I	2	3
Urea degradation II	1	3
Uridine-5′-phosphate biosynthesis	6	2
Valine biosynthesis	4	3
Valine degradation I	7	2
Valine degradation II	3	3
Very long chain fatty acid biosynthesis[8]	**4**	**1**
Wax esters biosynthesis II	2 (1)	4
Wound-induced proteolysis I	1	3
Xylitol degradation	2	3
Zymosterol biosynthesis	12	3

[1]Number of reactions in pathway. The number of reactions annotated/present in *A. fumigatus* Af293 is identical to this unless indicated otherwise by a bracketed figure. Some 'missing' reactions are spontaneous and do therefore not have an enzyme assigned. [2]Levels of annotation are as follows: 1 = manual annotation of entire pathway; 2 = automatic annotation followed by successful/predominantly successful manual hole filling; 3 = automatic annotated to completion/near completion; 4 = automatic annotation with holes unresolved. [3]References [13–15], [4][16–18], [5][19–23], [6][24], [7][25,26], [8][27].

Discussion

The mapping of metabolic and biosynthetic pathways of *A. fumigatus* Af293 represents the next step after genomic analysis in the understanding of the biology of this organism, and access to 242 pathways and 1576 reactions is now available. The level of annotation for a given pathway is chiefly dependent on the extent to which it has received attention; thus within this database, as with others, there are some pathways that are well attested, some for which there is less evidence, and others where their existence in *A. fumigatus* has yet to be demonstrated.

For the clinician with an interest in Aspergillosis, but without the expertise in genomics or fungal pathways, this resource provides a starting point for entry into *Aspergillus* biology, providing an answer to the question "How does *Aspergillus fumigatus* do that?" For researchers with more in-depth knowledge, the pathway resource will help advance *Aspergillus* biology: For instance, in mode-of-action screens for drug mechanisms, the pathways and

component genes that are implicated can be easily accessed from the outset, without having to mine the literature. Similarly, this resource will make it simpler to identify the fungal equivalents of human pathway components for comparisons which can be of value for better understanding of their function [28,29]. There are also features available in PathwayTools for comparison of the complete *A. fumigatus* pathway repertoire with that of other annotated genomes, enabling appreciation of similarities and differences compared to other fungi and humans.

In conclusion, the establishment of a curatable public facility for *A. fumigatus* Af293 pathways acts as the starting point for building a comprehensive resource and provides a tool comparable to that currently available for *Saccharomyces cerevisiae* (http://pathway.yeastgenome.org/) and *Candida albicans* (http://pathway.candidagenome.org/).

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