The genome of an industrial workhorse

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Sequencing of the filamentous fungus *Aspergillus niger* offers new opportunities for the production of specialty chemicals and enzymes.

Few microbes compare with the filamentous fungus *Aspergillus niger* in its ability to produce prodigious amounts of useful chemicals and enzymes. This fungus is the principal source of citric acid for food, beverages and pharmaceuticals[1] and of several important commercial enzymes, including glucoamylase, which is widely used for the conversion of starch to food syrups and to fermentative feedstocks for ethanol production. Although most of these fermentation processes are well established, the underlying genetics are still poorly understood. In this issue, Pel *et al.*[2] report the genome sequence of *A. niger* strain CBS 513.88. The availability of this sequence should provide invaluable aid toward improving the production of chemicals and enzymes in this organism.

Pel *et al.* sequenced tiled bacterial artificial chromosomes representing the entire *A. niger* genome to produce a high-quality assembly of 19 supercontigs with a combined length of 33.9 Mb. Approximately 5% of the genome corresponding to rDNA, telomeric repeats and short gaps were not sequenced, but the orientation of supercontigs on chromosomes was deduced in part by high synteny within centromere regions to related *Aspergillus* species[3–5]. Automated and manual curation was used to annotate a total of 14,165 genes.

Citric acid fermentation by *A. niger* has been studied for nearly 100 years[6], and modern day efficiencies are impressive compared with other fermentation processes, with routine yields of >200 g/l at >90% conversion of feedstock sugar into citric acid. Enzyme production is reportedly also very efficient, and although commercial yields are proprietary, glucoamylase secretion is known to occur at levels >10 g/l. In terms of total production of commercial enzymes, *A. niger* glucoamylase may be second only to *Bacillus* protease.

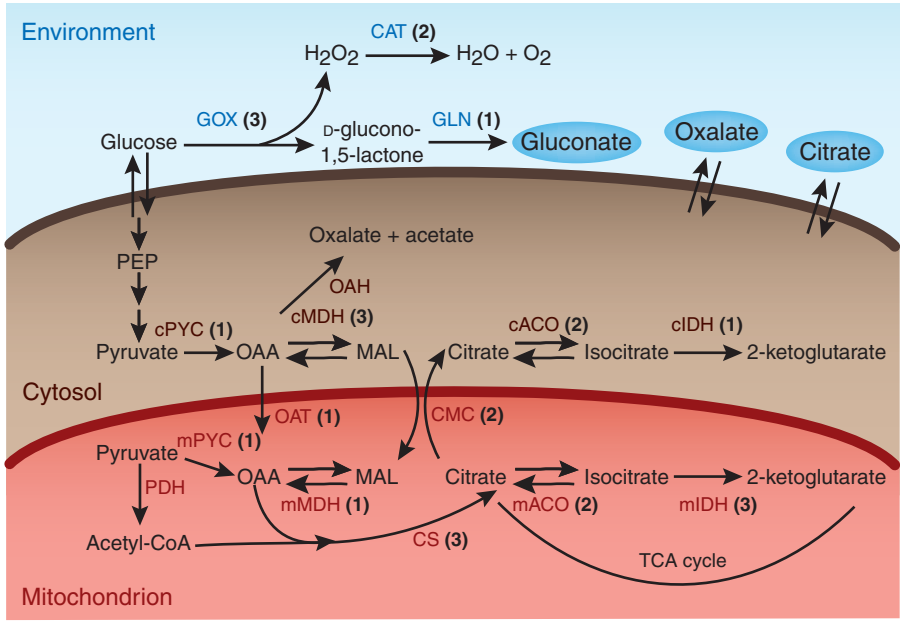

Figure 1 Central pathways and cellular locations for synthesis of gluconic, oxalic and citric acid by *A. niger*. Genetic redundancy, indicated by parenthetical numbering, may influence the high production levels. Abbreviations: GOX, extracellular glucose oxidase; GLN, gluconolactonase (only one of the four GLN-like sequences features a secretion signal); CAT, extracellular catalase; cPYC, cytoplasmic pyruvate carboxylase; OAH, oxaloacetase; cMDH, cytoplasmic malate dehydrogenase; cACO, cytoplasmic aconitase; cIDH, cytoplasmic isocitrate dehydrogenase; PDH, pyruvate dehydrogenase; mPYC, mitochondrial pyruvate carboxylase; OAT, oxaloacetate transporter; mMDH, mitochondrial malate dehydrogenase; CMC, citrate/malate carrier; mACO, mitochondrial aconitase; mIDH, mitochondrial isocitrate dehydrogenase; CS, citrate synthase.

To better understand the metabolic processes behind such high productivities, Pel *et al.* integrated the annotated genome data into a model of central carbon metabolism in *A. niger*[7]. The model incorporates the latest view of citrate synthesis, including the involvement of multiple malate dehydrogenase–like sequences, three of which encode cytoplasmic forms and one, a mitochondrial form (**Fig. 1**). Genetic multiplicity was further observed for citrate synthase (three genes) and aconitase (four genes, two cytoplasmic and two mitochondrial).

Interestingly, genetic redundancy was also observed among enzymes involved in the biogenesis of another commercially important organic acid, gluconic acid. In contrast to citrate, gluconic acid synthesis occurs extracellularly, with the first step catalyzed by glucose oxidase. The resulting d-glucono-1,5-lactone and hydrogen peroxide can be further metabolized by gluconolactonase and catalase,

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respectively. Putative genes encoding four glucose oxidases, four gluconolactonases and 11 catalases were detected, with several of these sequences featuring N-terminal signals typical of secreted proteins (**Fig. 1**). Whether such redundancy substantially influences organic acid production remains to be investigated.

A. niger possesses an impressive number of genes encoding carbohydrate-active enzymes, many of which participate in the degradation of plant polysaccharides. Categorized on the basis of structurally related catalytic domains (http://afmb.cnrs-mrs.fr/CAZY/) and compared with those of other fungal genomes, *A. niger*'s glycoside hydrolase and polysaccharide lyase genes are distributed in a way that reflects its ecological role.

As expected of a saprophyte often isolated from soils and decaying vegetation, an array of cellulase-, xylanase-, amylase- and pectinase-encoding genes were identified. Compared with related Aspergilli, few pectate lyase genes were predicted, but pectin lyases and polygalacturonases were abundant. Such interspecies diversity suggests different strategies in pectin degradation. In this connection, Pel *et al.* note that *A. niger* acidifies the substratum, a condition under which pectate lyases are less effective. The precise mechanism(s) involved in pectin degradation, particularly the roles and interactions among the 21 genes within glycoside hydrolase family 28, are under active investigation[8].

In addition to the organic acids and native enzymes, heterologous gene expression in *Aspergillus*[9] has been a focus of considerable research effort. A wide range of foreign proteins have been successfully expressed in *A. niger*, but relative to native enzymes such as glucoamylase, yields have been generally disappointing. Several components of the *Aspergillus* systems have been identified to better understand secretion pathways. Pel *et al.* describe several yeast and mammalian orthologs within the *A. niger* secretory pathway. Consistent with glycosylation processes commonly associated with extracellular proteins of *A. niger*, multiple glycosyltransferase genes were detected and their transcription verified using expression microarrays. These results provide a crucial framework for further research on the secretory machinery of *A. niger*.

The availability of genome data from related fungi facilitates comparative analyses of genome structure and evolution. Assembled or nearly complete genomes of nine *Aspergillus* species include important opportunistic human pathogens (for example, *A. fumigatus*), mycotoxin producers (for example, *A. flavus*) and essential experimental systems for genetic analysis (for example, *A. nidulans*)[3–5]. Within *A. niger*, careful comparisons of the genomes of the mutagenized CBS 513.88 strain, the unpublished wild-type strain ATCC1015 (ref.10) (http://genome.jgi-psf.org/Aspni1/Aspni1.home.html) and the extensive expressed sequence tags derived from conidial mutation strain ATCC 9029 (ref. 11) may accelerate strain improvement programs.

The *A. niger* genome illuminates challenges and opportunities for future research. Impressive genetic diversity, particularly among the carbohydrate-active enzymes, points toward novel enzymes of potential biotechnological significance. Identification and manipulation of components of the protein secretion machinery may improve yields of native and foreign proteins. Modeling central metabolism may one day lead to directed metabolic engineering and increased production of specialty chemicals and pharmaceuticals. Progress in these areas will rely on detailed genetic and biochemical analysis of individual genes together with high-throughput characterization of the metabolome, proteome and transcriptome.

COMPETING INTERESTS STATEMENT
The author declares that he has no competing financial interests.

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