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Review

What does genetic diversity of *Aspergillus flavus* tell us about *Aspergillus oryzae*?

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ABSTRACT

Aspergillus flavus and *Aspergillus oryzae* belong to *Aspergillus* section *Flavi*. They are closely related and are of significant economic importance. The former species has the ability to produce harmful aflatoxins while the latter is widely used in food fermentation and industrial enzyme production. This review summarizes the current understanding of the similarity of the *A. flavus* and *A. oryzae* genomes, the genetic diversity in *A. flavus* and *A. oryzae* populations, the causes of this diversity, and the relatedness of nonaflatoxigenic *A. flavus* strains to *A. oryzae*.

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Contents

1. Introduction

The genus *Aspergillus* represents groups of a very large number of asexual fungi (Fungi Imperfecti or Deuteromycetes) that are found in a broad range of habitats. Separation of individual species into various groups or sections was originally based on overlapping morphological or physiological characteristics (Raper and Fennell, 1965). *Aspergillus oryzae* and *Aspergillus flavus* belong to *Aspergillus* section *Flavi*. As with other fungi, these two species were conventionally distinguished from each other by morphological and cultural characteristics instead of by physiological or genetic traits (Jorgensen, 2007). *A. oryzae* has been widely used as the starter culture for the preparation of koji in the production of traditional Oriental fermented foods and alcoholic beverages, for example, soy sauce, miso, sake, and shochu. *A. oryzae* has also been an important source of many enzymes, such as glucoamylase, alpha-amylases and proteases used for starch processing, baking, and brewing worldwide (Machida et al., 2008). About two thirds of the bread production in the United States uses *A. oryzae* protease to release amino acids and peptides for yeast growth and gas production (Bigelis, 1992). The patented production of *A. oryzae* Taka-

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diastase, a neutral alpha-amylase, as a medicine in 1894 marked the beginning of modern enzyme biotechnology (Bennett, 1985). *A. oryzae* is capable of expressing high levels of heterologous enzymes. This ability led to the commercial production in *A. oryzae* of a recombinant lipase for use in detergents in 1988 by Novo Nordisk in Japan (Barbesgaard et al., 1992). *A. oryzae* and its fermentation byproducts are also used as probiotic and feed supplements for livestock (Lee et al., 2006b).

A. flavus, classified as a separate species but genetically almost identical to *A. oryzae*, is not used for commercial applications mainly due to its capability of producing aflatoxins (Scheidegger and Payne, 2003). *A. flavus* is thought to be predominately a saprophyte that grows on dead plant and animal tissue in the soil. Of all *Aspergillus*, it is the one most associated with preharvest contamination of certain crops. Because of its small spores and its ability to grow at 37 °C it can also be pathogenic to animals and humans. Infection by *A. flavus* has become the second leading cause of human aspergillosis (Krishnan et al., 2009). *A. flavus* can infect corn, peanuts, cotton, and nut trees as well as other crops and growth on these agricultural commodities often leads to contamination with aflatoxin B_1, a toxic and potent carcinogenic compound. Some researchers believe that *A. oryzae* is widely distributed in nature while others think that *A. oryzae* strains are just variants of *A. flavus* that have been domesticated through years of selection under artificial production environments. *A. oryzae* strains intended for commercial use commonly exhibit sparse sporulation, have floccose aerial mycelia and produce few or no sclerotia. The lack of these characteristics could be detrimental to dissemination and survival of *A. oryzae* in the field. However, there is evidence that certain nonaflatoxigenic *A. flavus* isolates obtained from the field have characteristics of *A. oryzae*. Therefore, *A. oryzae* may be a morphotypic variant of typical *A. flavus*.

Because of economic and food safety issues, *A. oryzae* continues to be classified as a separate taxon from *A. flavus*. The long history of safe use of *A. oryzae* by the food fermentation industry and lack of aflatoxin production has earned it GRAS (generally recognized as safe) status. In the past two decades, molecular biological techniques have been used to distinguish species in the *Aspergillus* section *Flavi*. These include restriction fragment length polymorphism (Klich and Mullaney, 1987), amplified fragment length polymorphism (Montiel et al., 2003), hybridization with aflatoxin biosynthetic genes (Klich et al., 1995), analysis of ribosomal DNA internal transcribed spacer regions (Kumeda and Asao, 2001), and single nucleotide polymorphisms (Lee et al., 2006a). In general, these methods are able to distinguish the *A. flavus*/*A. oryzae* group from the *A. parasiticus*/*A. sojae* group but do not separate *A. flavus* from *A. oryzae*. The goal of unambiguously distinguishing *A. oryzae* from *A. flavus* as a distinct species has not been realized. The unsuccessful endeavors further indicate the close relatedness of the two species.

A. flavus is not a monophyletic species. A morphological variant population has been identified. This variant, previously called *A. flavus* var *parvisclerotigenus* (Saito and Tsuruta, 1993) and distinguished by sclerotial size from "typical" *A. flavus*, has been reclassified as a separate species by Frisvad et al. (2005). Sclerotia are hardened masses of mycelia that serve as over-winter reproductive forms and remain dormant in soil until conditions are favorable for growth. The typical *A. flavus* isolates are called the L-strain whose average sclerotial size is greater than 400 μm while isolates of the variant strain called the S-strain have sclerotial size less than 400 μm (Cotty, 1989). Sclerotium morphology is a poor indicator of phylogeny. On laboratory growth media, when grown in the dark, S-strain isolates produce higher levels of aflatoxins, more abundant sclerotia, and fewer conidia than L-strain isolates. Like *A. oryzae*, attempts to distinguish S-strain *A. flavus* from typical *A. flavus* have not been accepted by all taxonomists. Nonaflatoxigenic S-strain isolates are very rarely found in natural environments or as crop contaminants (Orum et al., 1997).

Other species have been classified within section *Flavi* and five of these were found to produce aflatoxins. Two new species, *A. minisclerotigenes* and *A. arachidicola,* have been identified that produce both B- and G-aflatoxins but have sclerotia and conidia that resemble those of S-strain *A. flavus* (Pildain et al., 2008). Also some species within section *Nidulantes* and *Versicolores* make aflatoxin precursors and aflatoxins (Cary et al., 2009). Why some species are able to produce aflatoxins while others do not is not well understood and this distinction has important implications for human health and food safety.

2. Comparison of the *A. oryzae* and *A. flavus* genomes to those of other aspergilli

In December 2005, a consortium in Japan consisting of universities, institutions and the brewing industry led by The National Institute of Advanced Industrial Science and Technology released the genome sequence of *A. oryzae* RIB40 (ATCC 42149) (http://www.bio.nite.go.jp/dogan/MicroTop?GENOME_ID=ao). Later the genome sequence of *A. flavus* NRRL3357 was released by The Institute for Genomic Research (TIGR, Rockville, Maryland, USA, now J. Craig Venter Institute, JCVI) with funding from the Microbial Genome Sequencing Project to scientists at North Carolina State University (http://www.aspergillusflavus.org/genomics/). Together with studies on various aspects of the genetics of *A. oryzae* and *A. flavus*, these genome sequences have provided a wealth of information with regard to evolution and stability of the aflatoxin gene cluster as well as an assessment of the abilities of isolates of the same species and of different *Aspergillus* species to undergo recombination.

The assembled *A. oryzae* genome is about 37 Mb and organized in eight chromosomes. It is predicted to encode 12,074 proteins (Machida et al., 2005). The genome size is comparable to that of the closely related *A. flavus* NRRL3357, which is also about 37 Mb and consists of eight chromosomes (Payne et al., 2006). A comparative analysis of *A. oryzae* and *A. flavus* genomes revealed striking similarity between them. An array based genome comparison found only 43 genes unique to *A. flavus* and 129 genes unique to *A. oryzae*. Only 709 genes were identified as uniquely polymorphic between the two species (Georgianna and Payne, 2009).

To date, all other species in the genus *Aspergillus* whose genomes have been sequenced have eight chromosomes, but their genome sizes are smaller. The genome size of *A. fumigatus* is about 30 Mb, *A. nidulans* 31 Mb, *A. niger* 34–35 Mb, *A. terreus* 35 Mb and *A. clavatus* 35 Mb (NCBI Genome Projects, http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj). Comparison of the genome sequences of *A. fumigatus*, *A. nidulans*, and *A. oryzae* showed that the genomes of *A. fumigatus* and *A. nidulans* are predicted to encode 1412 and 2444 fewer proteins than the *A. oryzae* genome, respectively. Excluding singletons (genes without homologs in the fungal genome databases) the *A. oryzae* genome contains 16 and 26% more genes than the two species, respectively (Galagan et al., 2005). Syntenic analysis of the three aspergilli showed that common syntenic blocks and specific blocks are organized in a mosaic manner in the *A. oryzae* genome. Phylogenetic analysis with whole-genome data indicates that *A. nidulans* branched off from a common ancestor earlier than *A. oryzae* and *A. fumigatus*. Thus, the increase in the *A. oryzae* genome size likely is due to lineage-specific sequence expansion rather than loss of sequence in the *A. nidulans* and *A. fumigatus* genomes.

3. Origins of the extra genes in *A. oryzae* and *A. flavus* genomes

Machida et al. (2005) noticed that some genes from these non-syntenic blocks are paralogs of other orthologous genes that have a syntenic relationship among the aforementioned three aspergilli. In contrast to orthologs, which are genes from a common ancestor and encode proteins with the same function in different species, paralogs are