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Review

Food-Borne Fungi Kingdom

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Food-borne fungi may be divided into two categories, beneficial and detrimental. This has provided endless fascination and intrigue to those who have studied fungi, especially food-borne fungi. However, fungi are among the most versatile and diverse organisms in their morphology, life cycle, and ecology and the situation has made it difficult to understand fungal biology from the perspective of the broader fields of evolution, ecology, genetics, and population biology. Nowadays the population biology has come of age during the molecular revolution and this has influenced the choice of approaches and tools for food-borne fungi.

Characterization of food-borne fungi

Fungi are eukaryotes feed by adsorbing organic compounds from their environment and are generally composed of hyphae with cell walls. The non-motile organisms are recognized to reproduce by spores with one or several cells.

They are generally grouped in 4 division as follows due to similarity of structure, ribosomal DNA, cell wall composition, and lysine synthesis pathway. Of these, Deuteromycetes, unlike the other groups, has been well characterized on the basis of asexual reproduction (1, 2).

1) Zygomycetes
2) Ascomycetes
3) Deuteromycetes
4) Basidiomycetes.

Deuteromycetes has been further classified in 23 genera including *Acremoniu*, *Aspergillus*, *Botrti*, *Fusarium*, *Penicillium*, *Phoma*, *Paecilomyces*, *Scopulariopsis*, *Trichothecium*, *Trichoderma*, and *Wallemia* (3).

Aspergillus

Aspergillus species are mainly described and keyed out in many literatures, regardless of beneficial or detrimental. *Aspergilli* have been classified into 16 species, as shown in Table 1 (3).

Colonies usually growing rapidly, white, yellow, yellow-brown, brown to black or shades of green, mostly consisting of a dense felt of erect conidiophores (Fig. 1).

The distinction of this species from aflatoxigenic *A. flavus* is often difficult because of the many intergrading strains. Beneficial *A .oryzae* isolates differ from *A. flavus* by lighter green colonies and larger, less ornamented conidia, which are often not uniform in size.

Several species have attracted attention as human and animal pathogens or because of their

Table 1. Keys to the genera of *Aspergillus* species.

MEA, malt extract agar; Czapek, Czapek Dox agar medium. Handbook of Microbiological Media (Atlas, R.M. ed.) CRC Press (1993).

1a. Colonies white, black or in yellow, brown or grey colours 2
1b. Colonies in some shade of green..8

2a. Conidial heads white, often wet ....*A. candidus*
2b. Conidial heads yellow, some shade of brown or black 3

3a. Conidial heads dark brown to black ....*A. niger*
3b. Conidial heads not dark brown to black, but olive, yellow-brown or other shades of brown 4

4a. Conidial heads columnar, often cinnamon-brown to pinkish-brown ....*A. terreus*
4b. Conidial heads not columnar, colour yellow or brown 5

5a. Conidial heads olive to light brown; stipe brown. Hülle cells often produced ....*A. ustus*
5b. Conidial heads not olive; stipe hyaline or yellowish. Hülle cells absent 6

6a. Conidial heads pure yellow, conidia smooth to finely roughened ....*A. ochraceus*
6b. Conidial heads yellow-brown, conidia ornamented 7

7a. Conidia conspicuously ornamented with warts and tubercles, outer and inner wall can be distinguished..*A. tamarii*
7b. Conidia mostly roughened, outer and inner wall can not be distinguished..*A. wentii*

8a. Conidiophores typically brown, Hülle cells and *Emericella* teleomorph mostly present ....*A. nidulans*
8b. Conidiophores not typically brown, *Emericella* teleomorph absent............9

9a. Colonies on Czapek or MEA mostly restricted (colony diameter usually less than 1.5 cm within one week)..10
9b. Colonies growing faster with a diameter usually larger than 1.5 cm 11

10a. Colonies variably coloured, conidial heads biseriate, sometimes Hülle cells present..*A. versicolor*
10b. Colonies grey green, conidial heads uniseriate, on MEA or Czapek growing very restricted with poor sporulation, on low water activity media showing better development, Hülle cells not formed ....*A. penicillioides*

11a. Yellow *Eurotium* teleomorph produced in old cultures or on low water activity media ....*A. glaucus*
11b. Yellow *Eurotium* teleomorph absent...12

12a. Conidial heads yellow-green to dark yellow green 13
12b. Conidial heads blue to dark blue green 15

13a. Conidial heads predominantly uniseriate, conidia dark yellow green, conspicuously echinulate...*A. parasiticus*
13b. Conidial heads uni- and biseriate 14

14a. Conidia minutely echinulate, yellow green ....*A. flavus*
14b. Conidia irregularly roughened or smooth, greenish olive ....*A. oryzae*

15a. Conidial heads columnar, vesicles broadly clavate, conidia rough to echinulate ....*A. fumigatus*
15b. Conidial heads not columnar, vesicles narrowly clavate, smooth-walled...*A. clavatus*

ability to produce toxic metabolites. Others are important for their role in fermentation of oriental food products or industrial application in the production of organic acids or enzymes. The classification is mainly based on morphological characters (4, 5)

Fungal metabolites such as organic acids and enzymes are frequently employed in dairy, bakery and fermented food products, as well as in preservation. The fungi could also provide a direct source of protein. The beneficial forms may be purposely introduced or naturally present, while the detrimental ones appear as unwanted natural contaminants. In the last decades fungal contamination during processing and storage has attracted much attention, particularly because of the production of toxic metabolites. Although toxic fungi are ubiquitous and belong to the common contamination flora, their recognition is hampered by incomplete and often confusing literature.

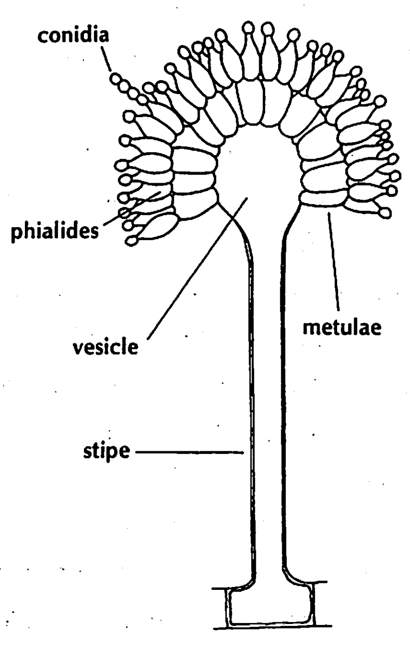

Fig.1. Typical morphological structure in *Aspergillus*

Aflatoxigenic Aspergilli

The profiles of secondary metabolites, including mycotoxins are of great use in systematics (4). *A. flavus* and *A. parasiticus* are well known for aflatoxigenic species (5). The presence of fungal growth on or in food products or in a Petri dish should be first confirmed directly with the naked eye and subsequently with a dissecting microscope. Fungal tissue (mycelium, fruit bodies, or sporulating structures) can be taken and subjected to examination and identification.

***Useful roles of Aspergilli* in food processing**

(a) Production of organic acids

Citric acid is the most important organic acid produced by fermentattion (5). Its annual production is estimated at 400,000 tons which are made mainly with *A. niger* (6). Citric acid is extensively used in the food industry as an acidualnt and flavoring substance.

Gluconic acid (50,000 tons/year) and its delta-lactone are applied in foods and in the medical field.

Itaconic acid can be made with *A. terreus* using molasses or wood hydrolysates, and finds application in the chemical industry.

(b) Detoxification of mycotoxins

Aflatoxin B_1 in some kinds of nut meals could be fully degraded during 7 days solid-substrate fermentation with a selected *Aspergillus* (7). Although the processes may be time consuming, biological detoxification of mycotoxins has the advantage over chemical and physical processes.

(c) Production of industrial enzymes

Within the range of enzymes produced by fermentation, the majority is hydrolytic enzymes, especially proteases. Proteases obtained from *Aspergillus oryzae* are applied in detergents, and in food processing (cheese ripening, bread making, and tenderization of meat).

Lipases synthesized by *Aspergillus* sp. are similar to pancreatic lipase in that they do not attack the 2-position in the triglyceride and show low specificity for the type of fatty acid whereas *Mucor miechei* lipase is active on short chain triglycerides with no marked positions specificity.

Starch-hydrolysing enzymes (alpha-amylases and glucoamylases) from *A. oryzae* and *A. niger* are applied in brewing and bread making.

Other hydrolases such as cellulases by *A. niger*, and pectinases by *Aspergillus* sp.are applied to improve digestibility of fibrous foods, and filterability of fruit juices and beer.

Fungi tend to secrete transeliminase type of pectinases that are specific for pectin (polymethy-lgalacturonate lyases).

(d) Transformation by protoplast fusion technique

Although any gene transfer technique can be used to construct high yielding strains, many fungal groups are said to lack a suitable system (7). For this organisms protplast fusion may hold the answer. If the cell wall is removed from fungi using enzymes in an isotonic medium, spherical proplasts result. In the fused product, if the two target chromosomes are largely homologous, recombination will occur.

Protoplast fusion offers the unique opportunity of bringing together two complete genomes and a very high frequency of recombinants can be obtained. Recently, one particularly successful host-vector system concentrated on employing both nitrate rductase (*nia*D) - deletion mutant: *A. oryzae* as a host and a potential chimera plasmid that directed highly effective promoter gene as a vector (8).

A homologous transformation system was developed which is applicable to analysis of the amylase gene promotor function in *A. oryzae* (9). The unique host-vector recombinant system may provide much of the impetus for recent developments in eukaryotic protein secretion and thus enable to the products to be made at such high levels that their recovery will become commercially feasible (10).

Finally, this unique system allows us to manufacture genetically modified organisms that might be accepted as more suitable food materials than those yielded by using bacterial host and plasmids bearing antibiotic-resistant genes, since they might cause undesirable state of systematic immunological responsiveness after their intake and digestion .

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