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Identification of clinically relevant aspergilli

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As the number of cases of aspergillosis grows, the number of species reported to cause the disease is increasing. Historically, classification and identification of aspergilli was accomplished using morphological characteristics. A number of molecular, immunological and biochemical methods are now available. For the most part, the results of the various approaches concur, yielding similar results in identifying aspergilli, so the 'best' method for identification is the method that best suits the needs of the researcher or clinician. Each identification method has advantages and disadvantages which will be discussed herein. The paper contains a listing of some of the available identification systems from those that address the whole genus to those capable of separating intraspecific strains.

Keywords *Aspergillus* identification keys

Advantages and disadvantages of major categories of identification methods

The morphological approach is the least expensive and quite accurate, but it requires use of a standardized plating and incubation regime, some skill with a microscope, and an understanding of the characteristics of the genus. The identification process usually takes a week, but can take longer, especially for some of the teleomorphic forms. All species descriptions of aspergilli are based, at least in part, on morphological characters. This is the only method available that includes all of the species in the genus.

Like the morphological approach, the biochemical approach requires culturing the fungi which takes about a week. The biochemical approach includes such things as the response of fungi to antibiotics or the production of certain biochemicals. The main limitation of the systems that rely on the production of certain biochemicals is that not all isolates of a species consistently produce the same metabolites. Although the use of inexpensive thin layer chromatography can yield good information for identification, metabolites can only be accurately identified with more sophisticated equipment such as high performance liquid chromatography (HPLC) and mass spectroscopy.

Immunological methods may be used on tissue samples, but are currently limited because they have been developed for only a few taxa. These methods have tremendous potential for providing rapid, accurate identifications and will probably play a larger role in both clinical and research laboratories in the future.

Molecular methods have the advantage of not necessarily requiring isolation or culturing of the fungi and require only a skilled technician for acquiring data. The equipment necessary for molecular work, however, is very expensive, accepted molecular methodologies change frequently, and the process needs to be overseen by a well-trained molecular biologist to avoid potential errors in identification. There is also the potential for sequencing a contaminating organism, thereby rendering a completely erroneous result.

Given that several systems of identification are currently available, care should be used to select methodologies that provide the level of identification required. Also, as the level of intraspecific variability is high in the aspergilli, species identification should employ systems created using several strains of each species. For maximum accuracy, a combination of identification methods should be used, and molecular identifications should be compatible with morphological features.

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When is specific and subspecific identification necessary?

In most clinical settings, immediate identification of the species causing a fungal disease is less important than rapid determination of the appropriate treatment regime most efficacious for the patient. This may not require identification of the isolate to the species level. Species level identification of a clinical isolate provides information on which to base empiric therapy, but isolates within a given species will vary in susceptibility to antifungal agents. What is really needed for patient treatment is probes /chemical tests etc. that will rapidly tell us: (a) that the patient does have a fungal infection, and, (b) which drugs will work on the specific fungal isolate infecting the patient. Such a system would reduce the need for a change in treatment when it is found that the specific strain is not responding to the initial therapeutic regimen. A system that does not require identification would also be unaffected by changes in the taxonomy of the fungi involved. Until such a system is fully developed and tested, fungal identification is necessary in the clinic.

The ability to differentiate among closely related species or strains within a species is sometimes important in disease prognosis, tracing nosocomial infections, epidemiological studies, litigation, etc. In these situations, accuracy of the identification is more important than the speed at which the identification is performed. In such cases, researchers frequently use more than one method of identification. Identification systems for strain differentiations should be designed to fit the specific need. Molecular methods are especially useful in distinguishing closely related species and fingerprinting strains within a species.

What are the clinically important aspergilli?

Of the nearly 200 species of *Aspergillus*, approximately 40 have been reported to cause mycoses (Table 1). The most commonly reported species in human aspergilloses are *A. fumigatus, A. flavus, A. terreus and A. niger* [1]. Not surprisingly, these are also the four most commonly reported species of *Aspergillus* in nature [2]. The vast majority of the aspergilli listed in Table 1 are common species that will grow at 37°C, however, species such as *A. deflectus* and *A. clavatonanicus* are relatively rare and *A. penicillioides* does not grow at 37°C [3,4]. Species causing aspergilloses are not restricted to just a few taxonomic sections of the genus. These facts indicate that aspergilloses are truly opportunistic infections, and virtually any *Aspergillus* species could cause disease under the right conditions. Any system used for general identification of clinical fungi should be fairly large and must include species not yet reported as etiologic agents.

Table 1 Clinically relevant aspergilli arranged by taxonomic section (approximate number of accepted species in section) [7,53,57,58]

Subgenus *Aspergillus*
Section *Aspergillus* (21) *Eu. chevalieri, Eu. herbariorum, Eu amstelodami Eu repens, Eu. rubrum*
Section *Restricti* (3) *A. penicillioides, A. restrictus, A. caesiellus*
Subgenus *Fumigati*
Section *Fumigati* (16) *N. fischeri, N. pseudofischeri, N. spinosa, A. fumigatus, A. lentulus*
Section *Cervini* (5)
Subgenus *Ornati*
Section *Ornati* (7)
Subgenus *Clavati*
Section *Clavati* (4) *A. clavatus, A. clavatonanicus*
Subgenus *Nidulantes*
Section *Nidulantes* (35) *Em. nidulans, Em. quadrilineata, A. unguis,*
Section *Versicolores* (24) *A. janus, A. sydowii, A. versicolor, A. granulosus*
Section *Usti* (5) *A. ustus, A. deflectus*
Section *Terrei* (1–3) *A. terreus*
Section *Flavipedes* (4) *A. carneus, A. flavipes, A. niveus*
Subgenus *Circumdati*
Section *Circumdati* (15) *A. ochraceus, A. sclerotiorum*
Section *Flavi* (17) *A. flavus, A. oryzae, A. alliaceus, A. tamarii, A avenaceus*
Section *Wentii* (6)
Section *Nigri* (15) *A. aculeatus, A. japonicus, A. niger, A. awamori*
Section *Candidi* (1) *A. candidus*
Section *Cremei* (6)
Section *Sparsi* (5)
Subgenus *Stilbothamnium* (5)
Subgenus *Ochraceoroseus* (2)

Identification systems

The following is a listing of some of the papers in which identification systems are presented, including author's name and date of publication in order to give an historical perspective to the list. It does not include those papers that do not present keys, probes/sequence data, tables of characters or some other method of differentiating among the species. It also does not contain references to systems which were unsuccessful in distinguishing among species under consideration. The notations on the 'general method used' refer to the method used in the identification system. In a number of studies information gathered by other methods was reported, but not used in the actual identification system.

General systems (with >30 species)

Raper & Fennell 1965 [3] – morphological. This was the last complete monograph of the genus.
Although many species have been described since then, the book is still used extensively.
Kozakiewicz 1989 [5] – morphological. In this book, strong emphasis is placed on the conidial characters as observed under scanning electron microscopy.
Klich 2002 [4] – morphological.
Pitt & Hocking 1997 [6] – morphological.
De Hoog *et al.* 2000 [7] – morphological.
Biolog 2005 (www.biolog.com [8]) – biochemical (Commercial Company).

Systems for specific sections of the genus (or major parts thereof)

Subgenus *Aspergillus*
Section *Aspergillus (Eurotium)* Blaser 1974/5 [9] – morphological; Gherbawy 2001 [10] – molecular.
Section *Restricti* – Pitt & Samson 1990 [11] – morphological; Tamura *et al.* 1996 [12] – biochemical.

Subgenus *Fumigati*
Fumigati – Frisvad & Samson 1990 [13] – biochemical (mostly anamorph); Yaguchi *et al.* 1994 [14] – morphological *Neosartorya* species; Someya *et al.* 1999 [15] – morphological *Neosartorya* species.

Subgenus *Clavati*
Section *Clavati* Tamura *et al.* 1999 [16] – biochemical and molecular.

Subgenus *Nidulantes*
Section *Nidulantes (Emericella)* – Christensen & Raper 1978 [17] – morphological; Horie 1980 [18] – morphological; Christensen & States 1982 [19] – morphological; Ismail *et al.* 1995 [20] – morphological; Horie *et al.* 1996 [21] – morphological; Zohri 2000 [22] – biochemical; Section *Versicolores* – Klich 1993 [23] – morphological.

Subgenus *Circumdati*
Section *Circumdati* – Christensen 1982 [24] – morphological; Varga *et al.* 2000 [25] – morphological & molecular.
Section *Cremei* – Christensen *et al.* 1964 [26] – morphological; Peterson 1995 [27] – morphological.
Section *Flavi* – Murakami 1971 [28] – morphological; Christensen 1981 [29] – morphological. Klich & Pitt 1988 [30] – morphological; Chang *et al.* 1995 [31] – molecular; Ito *et al.* 1999 [32] – morphological, biochemical & molecular; Kumeda & Asao 2001 [33] – molecular; Montiel *et al.* 2003 [34] – molecular.
Section *Nigri* Al-Musallam 1980 [35] – morphological; Yokoyama *et al.* 2001 [36] – molecular; Samson *et al.* 2004 [37] – morphological & biochemical; De Vries *et al.* 2005 [38] – molecular & biochemical; Gonzalez-Salgado *et al.* 2005 [39] – molecular.
Section *Wentii* – Peterson 1995 [27] – morphological.

Systems for closely related species pairs/groups

A. flavus/*A. oryzae* Murakami *et al.* 1969 [40] – morphological; Klich & Mullaney 1987 [41] – molecular; Lee et al 2004 [42] – molecular.
A. flavus/*A. parasiticus* Moody & Tyler 1990 [43] – molecular; Somashekar *et al.* 2004 [44] – molecular.
A. japonicus and aculeatus/*A. niger* Hamari *et al.* 1997 [45] – biochemical.
A. niger aggregate Varga *et al.* 1993 [46] – molecular.
A. niger/*tubingensis* Accensi *et al.* 1999 [47] molecular.
A. parasiticus/*A sojae* Murakami *et al.* 1982 [48] – morphological; Klich & Mullaney 1989 [49] – biochemical; Yuan *et al.* 1995 [50] – molecular.

Systems for specific and subspecific identification

A. caelatus DNA fingerprinting. McAlpin *et al*. 2005 [51] – molecular.
A. flavus Heinemann *et al.* 2004 [52] – molecular.
A. fumigatus see www.aspergillus.man.ac.uk [53] *A. fumigatus* in the database for references to the numerous methods of distinguishing strains of this species.
A. ochraceus Schmidt *et al.* 2003 [54] – molecular.
A. carbonarius Pelegrinelli *et al.* 2004 [55] molecular.

The future

Perhaps the most critical need in the area of 'identification' is the need for a system for rapid determination of drug susceptibility of clinical specimens. As outlined above, this system need not involve identification of species. There is also a need for new and improved identification systems. A new monograph of the genus should be written and it should include morphological, molecular, and biochemical data on each species as well as identification systems for all of the species. A public database using validated DNA sequences should be developed for molecular identification of aspergilli. Such a database is available for *Fusarium*

(http://fusarium.cbio.psu.edu [56]). Several sections of the genus have been taxonomically ignored for decades. These need to be rethought and reworked to include newly described species. Janos Varga and his colleagues have already made major progress in this arena, and one would hope that their work will continue. We are finding that ascospore characters are sometimes unreliable identification characters in the teleomorphic genera. Identification systems for these genera need to be revised to de-emphasized ascospore characters. Finally, a number of new species need to be formally described.

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