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The current role of *Aspergillus* and *Penicillium* in human and animal health

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Aspergillus and *Penicillium* are ubiquitous fungi, usually found as saprophytes. Only a few species are considered to be important in human or animal disease. However, many otherwise benign species are supreme opportunists and have been found increasingly as invaders of the immunocompromised. This paper first describes with a broad brush modern approaches to the classification of these genera, the reasons behind some name changes and the effective forces now acting to stabilize names. Recent taxonomic schemes are described. The taxonomy of pathogenic *Aspergillus* and *Penicillium* species is outlined, the subgenera where pathogens occur identified, and the question of why particular species are pathogens addressed. The significance of *Aspergillus* and *Penicillium* in mammalian disease is heightened by their production of potent mycotoxins. The importance of *Aspergillus flavus* and aflatoxins as a cause of human death in parts of Africa and Asia and the impact of ochratoxins, produced by *Penicillium verrucosum*, on human and animal health in Europe will be emphasized. Possible mycotoxin ingestion from spores poses a further health threat.

Aspergillus and *Penicillium* are universal fungi. Species of both genera are found almost everywhere on earth. *Penicillium* is dominant in cooler climates, especially the cool temperate zones, while *Aspergillus* is more common in the tropics. The great majority of species are saprophytes, commonly or occasionally found in soil, decaying vegetation, seeds and grains. Only a few well known species have been recognized as important pathogens of humans or domestic animals. *Aspergillus fumigatus* was described by Fresenius in 1863 from the bronchi and alveoli of a great bustard. It has been recognized for most of this century as a pathogen, capable of invading the lungs of humans, animals and birds. Generally, high concentrations of spores are necessary for infection. Healthy animals are able to ward off infections, so that severe illness usually results only from long-term exposure.

Several other *Aspergillus* species have a history of occasional pathogenicity. *Aspergillus terreus* is probably the most important. Although human infection is quite rare, the diseases caused are varied and not dissimilar to those caused by *A. fumigatus* [35]. *A. terreus* has recently become notorious in Australia and parts of the USA as a cause of fatal systemic infections in German Shepherd dogs or, rarely, other breeds [12, 31, 39]. *Aspergillus niger* has caused serious systemic infections, but is more commonly an aural invader. *Aspergillus flavus* is known as a rare cause of pulmonary disease in man, and is rather more common in birds. It has also been reported from skin, nails and occasionally systemic infections [4]. A few other species including the '*Aspergillus glaucus* group', *Aspergillus restrictus* and *Aspergillus sydowii* have been reported. However, infection by any of these species, with the exception of *A. fumigatus* and perhaps *A. terreus*, remains uncommon.

Penicillium species have long, and rightly, been regarded as benign, and of little or no consequence as human or animal pathogens. Only a few papers deal with

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Penicillium infections, but these have been reported in a remarkable range of sites. *Penicillium citrinum*, a common saprophyte, was isolated repeatedly from a urinary tract infection [21]. A systemic penicilliosis accompanying leukaemia was described by Huang & Harris [30]. Endophthalmitis due to *Penicillium chrysogenum* was reported by Eschete *et al.* [17]. *Penicillium* species have fatally invaded artificial heart valves [26, 70]; DelRossi *et al.* [13] reported successful management of such an infection. Wigney *et al.* [72] reported *Penicillium verruculosum* as the cause of osteomyelitis in a dog.

One *Penicillium* species, *Penicillium marneffei*, has been described as a true pathogen. Segretain, the great French medical mycologist, described *P. marneffei* as pathogenic to the Vietnamese bamboo rat [63] but also reported how he accidentally infected himself with an inoculating needle. A serious lesion resulted, which healed only slowly [64].

Until recently, the possible role of fungi in allergy was commonly neglected [1]. Species from both *Aspergillus* and *Penicillium* can undoubtedly be allergenic, but remarkably little information exists about the importance of particular species. A taxonomist is tempted to say that too often a rather simplistic view prevails that *Aspergillus* is *Aspergillus* and *Penicillium* is *Penicillium*. In consequence isolates from these genera have all too often not been identified to species level and so information necessary to build a picture of the important species is lost.

It appears unlikely that these genera are involved in allergy resulting from outdoor air. In one survey in Mexico City, *Penicillium* formed 14% of the spores collected, and *Aspergillus* only 1·7% [57]. However, the role of some species as a cause of allergy in indoor environments has been suggested quite frequently during the past 20 years. Conclusive evidence is still needed [18].

Some allergic responses certainly occur as a result of occupational contact with high levels of spores of particular species. Although 'farmer's lung' is usually considered to be due to actinomycetes, *A. fumigatus* also plays a role, as this species has a primary habitat in hay and heating feeds. 'Maltworker's lung' is a similar disorder resulting from the common presence of *Aspergillus clavatus* in barley during malting [56]. 'Cheese washer's disease' is another [62]. The potential hazard of mycotoxin ingestion associated with fungal spores will be discussed briefly later.

The recent and dramatic increase in numbers of people with immunodeficiencies has caused an equally dramatic increase in the incidence of infections with *Aspergillus* and *Penicillium* species. Aspergillosis is recognized as the most common fungal infection of immunocompromised patients [40]. *P. marneffei* has also been isolated a number of times from HIV-positive people in the past few years, in Europe, North America and elsewhere. Deng *et al.* [14] provided a comprehensive review. The first naturally occurring infection with *P. marneffei* was reported in about 1970; Deng *et al.* [14] recorded 20 more in the next 18 years. Alarmingly, from 1987 to 1992, Sirisanthana [66] reported 92 patients with *P. marneffei* infections in Chiang Mai Hospital, Chiang Mai, Thailand, alone. A high proportion of such infections are associated with immunocompromised people: in the report of Sirisanthana [66], 85 of 92 *P. marneffei* cases (93%) were HIV-positive.

A range of other species, some known, some unknown, have also been reported from AIDS patients. *Penicillium decumbens* was responsible for a systemic infection [2], while *P. chrysogenum* caused a fatal case of oesophagitis [29]. We have recently looked at a range of *Penicillium* isolates from various clinical sources, as described in more detail below.

Three fungal genera are of world importance for mycotoxin production: *Aspergillus* and *Penicillium* are two of them. It is easy to underestimate the significance of these two genera in human and animal health from this cause. Apart from the well recognized problem of mycotoxin ingestion from foods and feeds, the possibility of mycotoxin inhalation with fungal conidia also exists, and in the opinion of some authorities, constitutes a major problem. This paper will provide up-to-date information on these topics.

For various reasons, important changes in the taxonomy and nomenclature (systematics) of *Aspergillus* and *Penicillium* have occurred recently. While most of these changes do not directly affect the medical mycologist, it is important that the changes, and the reasons behind them, be understood. The good news is that most of the changes dictated by provisions of the International Code of Botanical Nomenclature (ICBN) have now been made. A period of stability in the species names in these genera can be expected. This topic will be dealt with first below.

Modern classification of *Aspergillus* and *Penicillium*

The last complete classification of *Aspergillus* and its sexual stages (now commonly called teleomorphs) was that of Raper & Fennell [53]. This was a practical classification, in almost exclusive use by those wishing to identify Aspergilli for the past 30 years. However, Raper and his colleagues often failed to consider key provisions of the ICBN. As Benjamin [8] wrote, the Raper & Fennell [53] classification's 'deficiencies are few, but are so fundamental that much of the taxonomy and nomenclature is involved. There is little conformance to the International Code of Botanical Nomenclature. Its taxonomy and nomenclature ... should be neither emulated nor condoned'.

The situation in *Penicillium* has been equally serious. The classification of Raper & Thom [54] was the accepted authority on this genus for 30 years and indeed is still in use in some countries. In its day it too was an outstanding taxonomic work, but it also suffered from lack of conformity to the ICBN.

The principal problems which Raper and his colleagues left as a legacy in both *Aspergillus* and *Penicillium* classification included: (i) a failure to observe priority and use the correct names under the ICBN; (ii) a failure to typify names, leading to uncertainty as to which taxon a name actually referred; (iii) failure to give precedence to teleomorph names, required under the ICBN; and (iv) division of these genera into 'Groups', a subgeneric classification with no status under the ICBN. Because later taxonomists recognized the need to follow the provisions of the ICBN, the result was confusion.

It is not my purpose here to describe the various proposals, techniques and agreements which have largely overcome these problems, as they have been adequately documented elsewhere [47, 49, 50]. However, the resultant changes are important.

(i). As dictated by the ICBN, names of the various teleomorph genera associated with *Aspergillus* and *Penicillium* take precedence over names of the same species in *Aspergillus* and *Penicillium*. It is recommended that *Eurotium* be used in place of the incorrect '*Aspergillus glaucus*' as a general term for this genus of very important spoilage fungi; *Neosartorya fischeri* is the correct name for the species Raper & Fennell [53] called *Aspergillus fischeri; Aspergillus nidulans* is properly known as *Emericella nidulans* when ascospores are present.

TABLE 1. The modern subgeneric classification of *Aspergillus*[a]

Subgenus	Section	Representative species	Teleomorph
Aspergillus	*Aspergillus*	*A. glaucus*	*Eurotium*
	Restrictus	*A. restrictus*	
Fumigati	*Fumigati*	*A. fumigatus*	*Neosartorya*
	Cervini	*A. cervinus*	—
Ornati	*Ornati*	*A. ornatus*	*Hemicarpenteles*, *Sclerocleista*
Clavati	*Clavati*	*A. clavatus*	—
Nidulantes	*Nidulantes*	*A. nidulans*	*Emericella*
	Versicolores	*A. versicolor*	—
	Usti	*A. ustus*	—
	Terrei	*A. terreus*	—
	Flavipides	*A. flavipes*	—
Circumdati	*Wentii*	*A. wentii*	—
	Flavi	*A. flavus*	—
	Nigri	*A. niger*	—
	Circumdati	*A. ochraceus*	*Petromyces*
	Candidi	*A. candidus*	—
	Cremei	*A. cremeus*	*Chaetosartorya*
	Sparsi	*A. sparsus*	

[a]Adapted from Klich & Pitt [32].

(ii). A consequence of the provisions of the ICBN is that if an *Aspergillus* or *Penicillium* name has been described inclusive of the teleomorph (e.g. *A. fischeri* Wehmer), this name cannot be used for the *Aspergillus* state alone. In consequence new anamorph names had to be introduced for a number of *Aspergillus* and *Penicillium* species with teleomorphs. Pitt [43] made the necessary changes in *Penicillium*, and Samson & Gams [59] for *Aspergillus*. For a more detailed discussion see [46].

(iii). As required by the rules governing priority in the ICBN, Pitt [43] necessarily changed a number of names when reclassifying *Penicillium*. He also complied with the provisions of the ICBN when he gave precedence to names in *Eupenicillium* and *Talaromyces* for *Penicillium* species which included teleomorphs. A simplified taxonomy, taking account of ICBN requirements and some important taxonomic changes since Pitt [43] was later published [44, 46].

(iv). Pitt [43] introduced a subgeneric and sectional classification for *Penicillium* to replace the improper 'Groups' of Raper & Thom [54]. Gams *et al.* [20] did the same for *Aspergillus*. As the *Aspergillus* changes are still not well known, the most important elements of this new classification of *Aspergillus* and teleomorphs are given in Table 1. The laboratory guide by Klich & Pitt [32] uses the new subgeneric and teleomorph names. Indeed this is the only nomenclaturally correct taxonomy of *Aspergillus* currently available.

During the 1980s, disagreements among specialists over species concepts in *Penicillium* compounded the existing confusion created by the change from the Raper & Thom to the Pitt classification. While this debate was very important and assisted in the clarification of many problems, it was counter productive because it created confusion for users of taxonomies. The realization that such disagreements were unacceptable led to the formation of the International Commission on *Penicillium* and *Aspergillus* (ICPA), a specialist commission under the Mycology Division of the International Union of Microbiological Societies (IUMS). The ICPA has the broad aim of reaching consensus on the systematics of these genera. Two specialist workshops in 1985 and 1989 [60, 61] assisted greatly in achieving that goal.

A still unsolved problem was that many names in *Aspergillus* perpetuated by Raper & Fennell [53] lacked priority, that is, they should be replaced by earlier, correct names. Development of a new classification, urgently required now, has not been possible because all specialists in the genus recognize that a reclassification in accordance with the provisions of the ICBN would mean abandoning many well known names such as *A. niger* and *Aspergillus ochraceus*, with further confusion resulting. True, it is now possible to conserve the names of economically important species under the ICBN, and indeed this has been accomplished for *A. niger* [19]. However, this ICBN provision does not apply to most species and conservation is a slow and time-consuming process.

A solution to this problem came from an unexpected quarter. Concerned at the apparently never-ending name changes in botany and mycology—so destructive to the whole system of naming plants and fungi—a group of experts conceived the idea of protecting names now commonly accepted but not necessarily correct under the ICBN. The concept which developed involved establishing lists of 'Names in Current Use' (NCU) and sanctioning those at a Botanical Congress. Although names in such lists would still be subject to most ICBN provisions, so that they could be combined, split and synonymized as usual, they would be protected from earlier names. A logical outcome of this approach would be a greatly improved stability in botanical and mycological names.

Recognizing the importance and indeed profound significance of this concept for *Aspergillus* and *Penicillium*, ICPA took the pioneering step of preparing an NCU list for the family *Trichocomaceae*, which includes *Aspergillus*, *Penicillium* and all their teleomorphs, for presentation at the International Botanical Congress in Yokohama, Japan in August, 1993. As events transpired, the concept of NCU lists was not accepted by the Congress, but scheduled for reappraisal at the next, to be held in St Louis in 1999. However, a resolution providing provisional protection for the *Trichocomaecae* list was passed at the Congress and will be written into the next ICBN. The net result is a substantial step forward: names contained in that list [51] have for practical purposes been cast in stone. Names of species in that list should be used in preference to any other names, regardless of priority. Some important examples: *A. ochraceus* has been given preference over *Aspergillus alutaceus*, a recently revived name which had already begun to inject instability; *Aspergillus nidulans* is now accepted as correct for the anamorph of *Emericella nidulans*, in place of the correct (but totally ignored) *Aspergillus nidulellus*; the controversy over species concepts for *Penicillium janthinellum* and *Penicillium simplicissimum* has been settled; and many early *Aspergillus* names in the literature can now be forgotten. Copies of the NCU list, which includes 614 species names in 27 genera, are available from the authors.

Although some discussion about the breadth and definition of some species in both genera will no doubt continue, I predict that names in use now, and accepted in the NCU, will only rarely be changed in the future.

TABLE 2. Common *Penicillium* species capable of growing at 37°C[a]

Subgenus	Series (Section)	Species
Aspergilloides	*Implicata (Aspergilloides)*	*P. chermesinum*
	Restricta (Exilicaulis)	*P. decumbens*
Furcatum	*Divaricata (Janthinella)*	*P. janthinellum*
	Furcatum (Oxalica)	*P. oxalicum*
		P. simplicissimum
Penicillium	*Penicillium (Expansa)*	*P. chrysogenum*
Biverticillium	*Simplicium (Miniolutea)*	*P. marneffei*
		P. minioluteum
		P. pinophilum
		P. funiculosum
		P. purpurogenum
	Simplicium (Islandica)	*P. islandicum*
		P. verruculosum

[a] From species listed in Pitt [46].

Pathogens and potential pathogens

What determines pathogenicity in genera such as *Aspergillus* and *Penicillium*? Do quite specific 'pathogenicity factors' exist, or are we looking simply at basic saprophytes which happen to possess physical characteristics conducive to pathogenic invasion? Much effort has been expended in seeking specific factors, but with little success. It still appears probable that the true pathogens, *A. fumigatus* and *P. marneffei*, possess pathogenicity factors which assist them. However, even in these cases much has to do with physical attributes.

The first point to be made is that the prime requirement for *Aspergillus* and *Penicillium* to be pathogenic in mammals or birds is the ability to grow at body temperatures. Obviously this point is not new, but it bears emphasis. In the case of mammals, 37°C is the magic figure; in the case of birds, somewhat higher. Although it is true that species incapable of growth at 37°C occasionally invade animal extremities, where temperatures are not so high, the likelihood of serious infection is much lower, and systemic infection impossible. It is a simple fact that a number of species of *Aspergillus* at best grow poorly at 37°C, and only about half of the known *Penicillium* species will grow at all.

In *Penicillium*, species in subgenus *Penicillium*, most species in subgenus *Furcatum* section *Furcatum* and most species in subgenus *Aspergilloides* will not grow at, or near, 37°C. Without invoking any other principle, it is clear that only a few *Penicillium* species classified outside the subgenus *Biverticillium* have the potential to be pathogens (Table 2). Perhaps growth at 37°C is the only requirement for a *Penicillium* species to be an opportunistic pathogen in mammals which lack normal defence mechanisms.

In *Aspergillus*, the majority of species are capable of growth at 37°C. However many of the accepted species in *Aspergillus* are rare, limiting the opportunities to invade. When such species are omitted, admittedly a rather arbitrary decision, the list of potential pathogens is as shown in Table 3. Note that the classification given is the currently accepted one, developed by Gams *et al.* [20], as given in Klich & Pitt [32].

While the listings given (Tables 2 and 3) are probably an oversimplification, it is the author's belief that virtually any species listed is capable, at some time or other, of

TABLE 3. Common potentially pathogenic species of *Aspergillus*[a]

Subgenus	Section	Species
Nidulantes	*Versicolores*	*A. caespitosus*
	Flavipides	*A. carneus*
		A. flavipes
		A. niveus
	Nidulantes	*A. nidulans*[b]
	Terrei	*A. terreus*
	Usti	*A. ustus*
Fumigati	*Fumigati*	*A. fumigatus*
		A. fischerianus[c]
Clavati	*Clavati*	*A. clavatus*
Circumdati	*Circumdati*	*A. ochraceus*
		A. alliaceus[d]
		A. auricomus
	Nigri	*A. niger*
		A. japonicus
		A. carbonarius
		A. foetidus
	Flavi	*A. flavus*
		A. parasiticus
		A. tamarii
	Candidi	*A. candidus*

[a] From species included in Klich & Pitt [32].
[b] Teleomorph *Emericella nidulans*.
[c] Teleomorph *Neosartorya fischeri*.
[d] Teleomorph *Petromyces alliaceus*.

causing illness in some individual who for whatever reason lacks the normal protective immunological defence mechanisms against fungal attack. Some *Aspergillus* and *Penicillium* species are very highly evolved and deserve their reputation as supreme opportunists. Moreover, their diversity in terms of biosynthetic capability is vast, leading to the important consequence that they are very difficult to inhibit chemically. Few antibiotic substances are known which will selectively affect species from these genera without major damage to host tissue.

The more common pathogenic species must possess characteristics other than growth temperature to provide them with a competitive advantage. For example, *P. marneffei* grows as a yeast phase at 37°C, a strange (indeed so far as is known, unique) character among species in these genera. The significance of this in terms of pathogenicity has apparently not been investigated, but clearly such growth would greatly assist dissemination within the blood stream. This is presumably the character which gives *P. marneffei* its edge. A second example concerns *A. terreus*, a species which causes fatal, disseminated infections in certain dogs. The reasons why dogs and specific breeds are affected are unknown, but probably lie in immunochemistry or specific attack mechanisms in the fungus. However, the mechanism by which dissemination occurs is clear. *A. terreus* produces aleuriospores under slightly oxygen-limiting conditions, such as beneath the surface of agar in Petri dishes [53]. Noting rapid dissemination in mice inoculated intravenously with *A. terreus* conidia, Pore & Larsh

TABLE 4. Species of *Aspergillus* producing conidia commonly less than 3 μm in diameter and able to grow at 37°C

Subgenus (Section)	Species	Conidial diameter (μm)[a]
Circumdati (Circumdati)	*A. auricomus*	2·5–3·0
	A. ochraceus	2·5–3·5
	A. sclerotiorum	2·5–3·0
Nidulantes (Flavipides)	*A. flavipes*	2·0–3·0
	A. niveus	2·5–3·5
	A. carneus	2·5–3·0
Nidulantes (Terrei)	*A. terreus*	2·0–2·5
Fumigati (Fumigati)	*A. fumigatus*	2·0–3·0
	A. fischerianus[b]	2·5–3·0

[a]Values from Klich & Pitt [32].
[b]Teleomorph *Neosartorya fischeri*.

[52] postulated that aleuriospores were responsible. Occasional later papers [39] have confirmed this, but most authors have not been aware of this highly specific mechanism for dissemination. Pore & Larsh [52] noted that *Aspergillus carneus, Aspergillus flavipes* and *Aspergilus niveus* also produce aleuriospores, but the significance of such spores in these species is uncertain.

We should indeed be glad that *A. fumigatus*, the best known *Aspergillus* pathogen, does not possess such aleuriospores. Why then is this species such an important pathogen? Primarily because its spores are drawn deep into the lungs.

As a number of mycologists have pointed out previously [3, 9, 11, 28] the ability of fungal spores to penetrate into the lungs depends primarily on spore size. *A. fumigatus* produces tiny conidia, little more than 2 μm in diameter, and these are capable of penetrating deep into the alveolar region. Given that this species is a marginal thermophile, with its primary habitat in heating vegetation, in which it grows very rapidly and sporulates prodigiously, the significance of *A. fumigatus* in diseases such as farmer's lung is clear, and its occurrence there needs no more complex explanation.

But why just *A. fumigatus*? What about other *Aspergillus* species? One important point is that few of these can both produce tiny conidia and grow at 37°C (Table 4).

One possible reason why potentially dangerous *Aspergillus* species such as *A. flavus* or *A. niger* are less frequently found as lung pathogens than *A. fumigatus* may be because their conidia are too large to penetrate below the level where the ciliated defensive lung cells can remove them [9]. However, as Campbell [9] also points out, conidial size is not the whole story. *A. clavatus* causes alveolar allergy [56], indicating deep penetration, but does not invade. Why does this difference exist? Two physical factors may be important. First, with an average size of 3·0–5·0 × 2·5–4·0 μm, the average volume of *A. clavatus* conidia is 16·6 μm^{-3}, more than 2·5 times that of *A. fumigatus* (average volume 6·1 μm^{-3} for conidia averaging 2·5 μm in diameter [32]). *A. fumigatus* is capable of deeper penetration. Second *A. clavatus* grows relatively slowly at 37°C. Based on physical factors, *A. fumigatus* should be the more serious lung threat.

A more important factor has recently emerged. Richard & DeBey [55] investigated the possibility that gliotoxin, a cytotoxic compound produced by *A. fumigatus*, might be

TABLE 5. Identity of some recent *Penicillium* isolates from human sources[a]

Strain number	Section	Identification	Source
FRR 3871	*Biverticillium*	*P. marneffei*	8-year-old child with agammaglobulinaemia
FRR 4059	*Biverticillium*	*P. marneffei*	Skin biopsy, AIDS patient
FRR 4079	*Biverticillium*	new species	Human tibial tissue
FRR 4173	*Biverticillium*	*P. piceum*	Human nail
FRR 4338	*Biverticillium*	new species	Lung washings, 12 months after heart-lung transplant
FRR 4339	*Furcatum*	new species	Lung infection by bronchoscopy
FRR 4341	*Talaromyces*	*T. thermophilus*	6-year-old girl with aplastic anaemia, left atrium wash
FRR 4390	*Furcatum*	*P. janthinellum*	Sputum fron non-Hodgkins lymphoma and respiratory dysfunction patient
FRR 4451	*Biverticillium*	*P. purpurogenum*	Lung washings
FRR 4463	*Biverticillium*	*P. minioluteum*	Drainage fluid, lung transplant
FRR 4489	*Talaromyces*	*T. macrosporus*	Vulval ulcer
FRR 4496	*Biverticillium*	*P. funiculosum*	Bronchoscopy, heart transplant

[a] Isolates studied at CSIRO Division of Food Science during 1992 and 1993. Most were from Australian sources; however FRR 4339 was from the USA and FRR 4341 from New Zealand.

produced during tissue invasion. Dr Richard recently announced that this was so, a significant discovery in our quest for understanding invasion. *A. fumigatus* is a successful human pathogen because it grows at 37°C, has tiny conidia, and produces gliotoxin to overcome host defence mechanisms.

From the above, two classes of pathogens can be seen. The first broad class has as its principal invasive feature the ability to grow at 37°C, permitting entry to tissue from wounds or other adventitious entry points, especially in the immunocompromised. The second, smaller class, consists of species which not only grow at 37°C, but have conidia small enough to penetrate the lungs of mammals or birds.

Penicillium pathogens observed recently

During the past 2 years, we have identified several *Penicillium* species from pathogenic situations which emphasize the points about pathogenic potential made previously. The sources and identifications of these isolates are given in Table 5.

Several points are important. First, the wide range of species found: the 12 isolates belong to 11 species. Second, 10 of the 12 isolates are from *Penicillium* subgenus *Biverticillium* or teleomorphs associated with that subgenus. Third, three new species were present, one similar to *P. janthinellum*, the second with a resemblance to *Penicillium piceum*, an uncommon species in subgenus *Biverticillium*, and the third near *Penicillium funiculosum*, but with unusual synnematous structures developing in age. Fourth, two species of *Talaromyces* were found, both worthy of comment. *Talaromyces macrosporus*, regarded by Pitt [43] as a variety of the common species *Talaromyces flavus*, but now given species status [51], is a fungus important in food spoilage, but rarely isolated from sources other than heat-processed fruit juices. Pathogenicity has never been indicated and its isolation from a vulval ulcer is remarkable. The isolation of

Talaromyces thermophilus from a child's heart is equally noteworthy: this species is thermophilic and also rarely isolated. Its main sources to date have been soil and heated vegetation such as compost and hay [43]. The *Talaromyces* state of this species has rarely been seen in culture. This isolate produced the *Talaromyces* teleomorph on malt extract agar containing millet seed after 6 weeks at 45°C.

Mycotoxins

No discussion of the role of *Aspergillus* and *Penicillium* in human and animal health would be complete without reference to mycotoxins. Indeed the formation of mycotoxins by these genera is undoubtedly the most significant of their contributions to mammalian ill health. Two of the four most significant mycotoxins in the world are produced by these genera: aflatoxins by *A. flavus* and closely related species, and ochratoxin A by *Penicillium verrucosum*. The intention here is not to comprehensively review these mycotoxins and the problems they cause, but to provide an up-to-date summary of current understanding.

Aflatoxins. After three decades of research, aflatoxins continue to dominate current work on mycotoxins. One independent source has estimated that $100m has been spent on aflatoxin research in North America alone, with little impact on its incidence.

Research on aflatoxins in recent years has been broadly based: major areas include analytical methods, epidemiology, international control and prevention or minimization of production in susceptible crops such as peanuts and corn.

Central to studies on epidemiology has been the long-standing question of the relative importance of the hepatitis B virus vs. aflatoxins as the leading cause of human liver cancer. This has continued to be hotly debated in recent years. A major advance has been the development of analytical techniques sufficiently sensitive to measure aflatoxin levels in human blood or urine, and hence to directly assess aflatoxin intake by a population. This is a great advance over previous intake estimates made by monitoring aflatoxin levels in food supplies [41, 42, 65]. The new techniques include aflatoxin–albumin adducts from human sera [6, 7, 58, 73, 74], immunoassays on sera [15] or urine [27] and competitive radioimmunoassays on aflatoxin–DNA adducts [23] or aflatoxin–guanine adducts [5] in urine. In the very large and thorough study by Groopman *et al.* [23], a high correlation was found between aflatoxin intake on one day and the level of aflatoxin–DNA adduct in urine on the next. The assay method of Autrup *et al.* [7] was sufficiently sensitive to detect increased levels of aflatoxin–serum albumin adducts in workers in a feed mill working with raw materials containing low ($26\,\mu g\ kg^{-1}$) levels of aflatoxins.

Results from some of these studies are very interesting. Autrup *et al.* [6, 7] suggested that an observed higher incidence of liver cancer in feed mill workers in Denmark might be partly explained by levels of aflatoxin in raw materials handled by such workers. Evidence remains circumstantial, however. In areas with high liver cancer incidence (e.g. in parts of Kenya), Autrup *et al.* [5] concluded that aflatoxin B_1 levels showed a moderate correlation with liver cancer, while hepatitis B infection rates were uncorrelated. Hatch *et al.* [27] studied eight locations in Taiwan which covered the range of incidence of liver cancer. Urinary aflatoxin levels showed a very high variation (500-fold) which correlated well with liver cancer levels in various localities. The correlation with hepatitis B incidence was much lower, consistent with the view that aflatoxin is involved in human liver cancer in Taiwan. In a cohort study on 7900 men in Guangxi province, China, Yeh *et al.* [75] showed a very high correlation between place of residence, estimated aflatoxin intake and liver cancer. However, no correlation was observed between hepatitis B virus incidence and liver cancer.

Wild *et al.* [74] showed a wide variation in aflatoxin–albumin adduct levels in Africa, Thailand, Nepal, China and Europe. Levels in Gambia, Senegal and Nigeria (all West African countries) were particularly high, and these areas have very high rates of liver cancer. Although liver cancer rates in Thailand are also high, levels of aflatoxin intake are much lower and do not correlate with cancer rates [67]. Liver cancer in Thailand is frequently attributable to other causes such as liver fluke [68].

Surveys of the incidence of aflatoxins in Southeast Asian foods recently carried out in my laboratory have shown that the levels of aflatoxins in Thai commodities, while significant and probably excessive by Western standards, are not sufficiently high to cause widespread cancer. This finding was in good agreement with the blood sampling data of Wild *et al.* [74]. However, the same survey showed that the incidence of aflatoxins in Indonesian peanuts was quite excessive, unacceptable by any standards and undoubtedly responsible for deaths from liver cancer.

Current opinion is that cancers produced by aflatoxin, by hepatitis B, and by some other agents (e.g. Southeast Asian parasites), are distinct and can be distinguished histologically. Hence their relative contributions can be quantified. The most recent opinion is that a synergy exists between aflatoxin and hepatitis B, and that perhaps a factor of 15 applies to populations exposed to both agents. In other words, the risk of liver cancer from an exposure to a given level of aflatoxin is perhaps 15 times as high in countries where hepatitis B virus is endemic.

Attempts to control aflatoxins in international trade, centre on the Codex Alimentarius Commission of the World Health Organization. An alarming trend continues: European countries, with no endemic aflatoxin problems, continue to press for unrealistically and unnecessarily low limits on aflatoxins in foods in international trade, while producer countries in tropical regions continue to deny they have a problem with aflatoxin, thus failing to provide neutral Codex countries with information which might halt such unrealistic demands. Publication of data showing the extent of aflatoxin occurrence in Southeast Asia, for example, continues to be a sensitive political issue. Prolonging the time during which aflatoxins will continue to kill people is an inevitable result of these attitudes.

Attempts to control aflatoxin at source continue. Research is centred on understanding the aflatoxin biosynthetic pathways, on the development of resistant cultivars of susceptible crops, on the development of plant genes which will impart resistance to *A. flavus* invasion, and on the use of non-toxigenic *A. flavus* or *Aspergillus parasiticus* as a competitor to the naturally occurring toxigenic strains. My personal viewpoint is that only the latter will yield results in the short term.

Ochratoxin A. Ochratoxin A is a nephrotoxin, originally described from *A. ochraceus* and closely related species. Little evidence exists, however, that *Aspergillus* species are an important source of ochratoxin, except perhaps in some tropical areas [48]. Kidney diseases (nephritis) in Scandinavian pigs have long been known and the possibility that this disease was due to ochratoxin A was postulated more than 20 year ago [34]. Ochratoxin A has more recently been found to be of very common occurrence in pig kidneys in Europe [24]. Links between pigmeat consumption and human nephropathy in Scandinavia and other parts of northern Europe were suggested more than 15 years ago [33]. Recent studies indicate that ochratoxin A is also commonly found in human blood samples in Europe [25]. The indication that ochratoxin A may be responsible for renal damage, renal failure and the premature death of individuals in large parts of northern and central Europe cannot be taken lightly. The source of ochratoxin A in Europe is the common fungus *P. verrucosum* [45]. This species is endemic in barley, the major feed ingredient for most pigs raised in northern and central Europe. The ochratoxin consumed in feed is stored in the depot fat of the pigs, from where it enters the human system.

The problem of ochratoxin A in Europe generally continues to be unrecognized outside scientific circles. Scientists have been privately stating for years that levels of ochratoxin A in Europe are too high, but little public awareness exists even now. Alarm in official circles in one European country has reached the point where officialdom is seriously considering testing each individual pig at slaughter for ochratoxin A levels in blood and destroying those which fail to meet acceptable levels.

Lack of knowledge of the causes of this problem continue to astound. In a recent publication intended to be an authoritative source of information on nephrotoxins, only one of 15 chapters correctly identified *P. verrucosum* as the source of ochratoxin A in Europe! Knowledge of the causal system also continues to be limited. No information appears to have been published on when the *P. verrucosum* enters the barley and under what conditions the ochratoxin is formed. A logical guess is that, like *A. flavus* in peanuts, *P. verrucosum* will be found to be a systemic pre-harvest invader of barley, perhaps normally present, and the expression of ochratoxin will depend primarily on climatic factors at, or before, harvest. As with aflatoxin, eventual control of the incidence of ochratoxin A will stem from detailed knowledge of the fungus and its relationship with the crop on which it grows.

Other nephrotoxin problems exist in Europe, perhaps again caused by *Penicillium* species, which clearly grow more aggressively in the cooler climatic conditions of Europe than in the tropics, USA or Australia. The problem of Balkan endemic nephropathy has neither gone away nor been solved [10]. However, attention in informed circles is swinging away from ochratoxin A as a potential cause, towards uncharacterized toxins produced by the closely related species *Penicillium aurantiogriseum* [36–38].

Mycotoxins in spores. Mycotoxicologists have recently drawn attention to the possibility that significant levels of mycotoxins may be absorbed from inhaled spores [18, 69]. The problem is likely to be most serious with aflatoxins, which are known to be produced at high concentration in the conidia of *A. flavus* and *A. parasiticus* [71]. Two recent case reports indicate that fungal spores inhaled in sealed granaries have caused human illness. In one case, a female farm worker suffered acute renal failure, which resolved in 24 days, after working for 8 h in a granary which had been closed for several months. Inhalation of ochratoxin in *A. ochraceus* spores was implicated [16]. In the other, a young man developed a neurological disorder after clearing mouldy silage from a silo [22]. Although no specific fungus was implicated, the symptoms suggested *Penicillium* neurotoxins. No doubt other such cases exist, but are generally unrecognized.

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