

## A reappraisal of fungi producing aflatoxins

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### Abstract

Aflatoxins are decaketide-derived secondary metabolites which are produced by a complex biosynthetic pathway. Aflatoxins are among the economically most important mycotoxins. Aflatoxin B<sub>1</sub> exhibits hepatocarcinogenic and hepatotoxic properties, and is frequently referred to as the most potent naturally occurring carcinogen. Acute aflatoxicosis epidemics occur in several parts of Asia and Africa leading to the death of several hundred people. Aflatoxin production has incorrectly been claimed for a long list of *Aspergillus* species and also for species assigned to other fungal genera. Recent data indicate that aflatoxins are produced by 13 species assigned to three sections of the genus *Aspergillus*: section *Flavi* (*A. flavus*, *A. pseudotamarii*, *A. parasiticus*, *A. nomius*, *A. bombycis*, *A. parvisclerotigenus*, *A. minisclerotigenes*, *A. arachidicola*), section *Nidulantes* (*Emericella astellata*, *E. venezuelensis*, *E. olivicola*) and section *Ochraceorosei* (*A. ochraceoroseus*, *A. rambellii*). Several species claimed to produce aflatoxins have been synonymised with other aflatoxin producers, including *A. toxicarius* (= *A. parasiticus*), *A. flavus* var. *columnaris* (= *A. flavus*) or *A. zhaoqingensis* (= *A. nomius*). Compounds with related structures include sterigmatocystin, an intermediate of aflatoxin biosynthesis produced by several *Aspergilli* and species assigned to other genera, and dothistromin produced by a range of non-*Aspergillus* species. In this review, we wish to give an overview of aflatoxin production including the list of species incorrectly identified as aflatoxin producers, and provide short descriptions of the 'true' aflatoxin producing species.

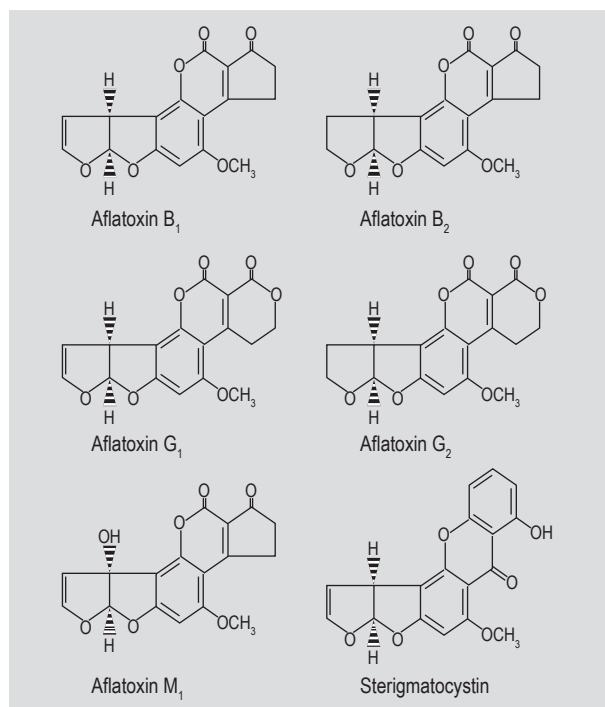
**Keywords:** aflatoxin production, *Aspergillus*, sterigmatocystin

### 1. Introduction

Aflatoxins are the most thoroughly studied mycotoxins. In the early sixties, toxicity of animal feeds containing contaminated peanut meal led to the death of more than 100,000 turkeys from acute liver necrosis (turkey X disease; Blout, 1961; Sargeant *et al.*, 1961; Nesbitt *et al.*, 1962; Van der Zijden *et al.*, 1962). Scientists identified the toxin-producing fungus as *Aspergillus flavus*, and the toxic agents as a group of structurally related difuranocoumarins that were named as aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> based on their fluorescence under UV light (blue or green) and relative chromatographic mobility during thin-layer chromatography (Figure 1). Aflatoxin B<sub>1</sub> is the most potent natural carcinogen known

(Squire, 1981) and is usually the major aflatoxin produced by toxigenic strains. Apart from those mentioned above, over a dozen other aflatoxins including aflatoxins P<sub>1</sub>, Q<sub>1</sub>, B<sub>2a</sub> and G<sub>2a</sub> have been described, especially as mammalian biotransformation products of the major metabolites (Heathcote and Hibbert, 1978), while aflatoxin D<sub>1</sub> was detected in ammoniated corn (Grove *et al.*, 1984), and aflatoxin B<sub>3</sub> as a metabolite of *A. flavus* (Heathcote and Dutton, 1969). Aflatoxin M<sub>1</sub>, a hydroxylated metabolite is found primarily in animal tissues and fluids (milk and urine) as a metabolic product of aflatoxin B<sub>1</sub> (Figure 1).

Many substrates support growth and aflatoxin production by aflatoxigenic moulds; natural contamination of cereals,



**Figure 1. Structures of the most important aflatoxins and sterigmatocystin.**

figs, oilseeds, nuts, tobacco, and a long list of other commodities occurs commonly (Bennett and Klich, 2003). These mycotoxins most frequently contaminate peanut, corn and cereals, but also occur in meat, milk (aflatoxin M<sub>1</sub>) and eggs of animals that have consumed contaminated feeds.

Aflatoxin B<sub>1</sub> exhibits hepatocarcinogenic and hepatotoxic properties, and is referred to as the most potent naturally occurring carcinogen. The International Agency for Research on Cancer has classified aflatoxin B<sub>1</sub> as a group I carcinogen (IARC, 1982). The diseases caused by aflatoxin consumption are called aflatoxicoses. Acute aflatoxicosis is produced when moderate to high levels of aflatoxins are consumed. Acute episodes of disease symptoms may include haemorrhage, acute liver damage, oedema, alteration in digestion, absorption and/or metabolism of nutrients, and may result in death. Acute aflatoxicosis epidemics occurred in India in 1974, resulting in 397 recognised cases and 106 deaths (Krishnamachari *et al.*, 1975), in Kenya in 1981 (Ngindu *et al.*, 1982) and in 2004 and 2005 causing more than 150 deaths (Azziz-Baumgartner *et al.*, 2006; Lewis *et al.*, 2005). Acute hepatitis associated with consumption of mouldy grains has also been reported in other areas in Africa, India and Malaysia (Chao *et al.*, 1991; Coulter *et al.*, 1986; Lye *et al.*, 1995; Patten, 1981). Chronic aflatoxicosis results from prolonged ingestion of low to moderate levels of aflatoxins. The effects are usually subclinical and difficult to recognise. Some of the common symptoms are impaired food conversion and slower rates of growth with or without the production of an overt aflatoxin syndrome. Chronic

aflatoxicosis results in cancer and immune suppression and other 'slow' pathological conditions (Eaton and Groopman, 1994). The liver is the primary target organ, with liver damage occurring when poultry, fish, rodents, and nonhuman primates are fed aflatoxin B<sub>1</sub>. There are substantial differences in species susceptibility. Aflatoxicosis has been observed in various animals including birds, dogs and other wild and domesticated animal species (Eaton and Groopman, 1994; Newman *et al.*, 2007).

Aflatoxins are decaketide-derived secondary metabolites which are produced by a complex pathway involving over 16 steps after the synthesis of the first stable intermediate, norsolorinic acid. In contrast to most polyketide synthases, the starter unit for aflatoxin biosynthesis is hexanoate, which is produced by a fatty acid synthase (Hicks *et al.*, 2002; Hitchman *et al.*, 2001). Sterigmatocystin, a related dihydrofuran toxin, is a late metabolite in the aflatoxin pathway and is also produced as a final biosynthetic product by a number of species such as *Aspergillus versicolor* and *Emericella nidulans*. Sterigmatocystin is both mutagenic and tumorigenic but is less potent than aflatoxin (Berry, 1988; Figure 1). Biosynthetic genes for aflatoxin pathway enzymes from *A. flavus* and *A. parasiticus* show high sequence similarity to the sterigmatocystin pathway genes of *E. nidulans* (Brown *et al.*, 1996; Payne and Brown, 1998). In *A. nidulans*, the sterigmatocystin gene cluster is about 60 kbp long and comprises 25 genes, the transcription of which is regulated by a Zn(II)2Cys6 DNA binding protein encoded by the *aflR* gene. The functions of the gene products identified so far include the fatty acid synthase and polyketide synthase mentioned earlier, five monooxygenases, several reductases, dehydrogenases, a methyltransferase, and an esterase (Brown *et al.*, 1996). The *A. flavus* and *A. parasiticus* aflatoxin gene clusters are about 70 kbp long, and consist of at least 24 different genes (Yu *et al.*, 1995, 2004). The *A. flavus* cluster is 96% identical to that of *A. parasiticus* and 91% identical to that of *A. nomius*. Coding regions generally have 4-10% higher sequence identity than intergenic regions (Cary and Ehrlich, 2006). In the recent years, considerable efforts have been made to understand the genetics and molecular biology of aflatoxin biosynthesis (Bhatnagar *et al.*, 2003; Chang *et al.*, 2007; O'Brian *et al.*, 2007; Yu *et al.*, 2002, 2004, 2007).

## 2. Aflatoxin producing species

The list of species that have been (incorrectly) reported to produce aflatoxins is very long; several species have been reported to be able to produce this metabolite (Tables 1 and 2). None of these species produce aflatoxins, and many of these names are not accepted as valid species in any case. The reports on aflatoxin-producing abilities of *A. terreus* could be due to the fact that territrems reveal blue fluorescence under long-wave ultraviolet (UV) light and have retention values similar to that of aflatoxin B<sub>1</sub> on

Table 1. *Aspergillus* species incorrectly reported to produce aflatoxins.

Species	Reference
<i>Aspergillus</i> section <i>Aspergillus</i>	
<i>A. glaucus</i>	Hanssen and Jung, 1973; Samajpati, 1979
<i>Eurotium amstelodami</i>	Janicki <i>et al.</i> , 1972; Abarca <i>et al.</i> , 1997
<i>Eurotium chevalieri</i>	Mabrouk and El-Shayeb, 1980; Kulik and Holaday, 1966; Leitao <i>et al.</i> , 1989; El-Kady <i>et al.</i> , 1994; Abarca <i>et al.</i> , 1997
<i>E. intermedium</i>	Kulik and Holaday, 1966; Leitao <i>et al.</i> , 1989; El-Kady <i>et al.</i> , 1994
<i>Eurotium herbariorum</i>	Vázquez-Belda <i>et al.</i> , 1995
<i>Eurotium repens</i>	Kulik and Holaday, 1966; Janicki <i>et al.</i> , 1972; Leitao <i>et al.</i> , 1987, 1989; Abarca <i>et al.</i> , 1997
<i>Eurotium rubrum</i>	Abarca <i>et al.</i> , 1997; Leitao <i>et al.</i> , 1987, 1989; Kulik and Holaday, 1966
<i>Aspergillus</i> section <i>Candidi</i>	
<i>A. candidus</i>	Abarca <i>et al.</i> , 1997; Jayaraman and Kalyanasundaram, 1980; Samajpati, 1979
<i>Aspergillus</i> section <i>Circumdati</i>	
<i>A. ochraceus</i>	Van Walbeek <i>et al.</i> , 1968; Reddy <i>et al.</i> , 2004
<i>A. ostianus</i>	Scott <i>et al.</i> , 1967
<i>A. sulphureus</i>	Scott <i>et al.</i> , 1970; Barr and Dawney, 1975
<i>Aspergillus</i> section <i>Cremeri</i>	
<i>A. wentii</i>	Schroeder and Verrett, 1969; Kulik and Holaday, 1966; De Waart <i>et al.</i> , 1975
<i>Aspergillus</i> section <i>Flavi</i>	
<i>A. oryzae</i>	El-Hag and Morse, 1976; Adebajo, 1992; El-Kady <i>et al.</i> , 1994; Abdel-Mallek <i>et al.</i> , 1993; De Waart <i>et al.</i> , 1975; Atalla <i>et al.</i> , 2003; Drusch and Ragab, 2003; Basappa <i>et al.</i> , 1967; Samajpati, 1979; Boller and Schroeder, 1966
<i>A. tamarii</i>	Lalithakumari and Govindaswami, 1970; El-Kady <i>et al.</i> , 1994; Goto <i>et al.</i> , 1996, 1997; Klich <i>et al.</i> , 2000
<i>A. terricola</i>	Moubasher <i>et al.</i> , 1977
<i>Aspergillus</i> section <i>Fumigati</i>	
<i>A. fumigatus</i>	Sodhi <i>et al.</i> , 1985; Abarca <i>et al.</i> , 1997; Pepeljnjak <i>et al.</i> , 2004
<i>Aspergillus</i> section <i>Nidulantes</i>	
<i>A. versicolor</i>	Masimango <i>et al.</i> , 1977; Atalla <i>et al.</i> , 2003
<i>Emericella nidulans</i>	Janicki <i>et al.</i> , 1972; Hanssen and Jung, 1973; Ahmed <i>et al.</i> , 2005
<i>Emericella rugulosa</i>	Schroeder and Kelton, 1975
<i>Aspergillus</i> section <i>Nigri</i>	
<i>A. niger</i>	Kulik and Holaday, 1966; Janicki <i>et al.</i> , 1972; Masimango <i>et al.</i> , 1977; Glinsukon <i>et al.</i> , 1979; Sodhi <i>et al.</i> , 1985; Ibrahim <i>et al.</i> , 1990; Waghray <i>et al.</i> , 1988; Reddy <i>et al.</i> , 2004
<i>A. ficuum</i> , <i>A. carbonarius</i> , <i>A. japonicus</i>	Masimango <i>et al.</i> , 1977
<i>Aspergillus</i> section <i>Restricti</i>	
<i>A. restrictus</i>	Samajpati, 1979
<i>Aspergillus</i> section <i>Terrei</i>	
<i>A. terreus</i>	Sripathomswat and Thasnakorn, 1981; Abarca <i>et al.</i> , 1997; Atalla <i>et al.</i> , 2003
<i>Aspergillus</i> section <i>Zonati</i>	
<i>A. zonatus</i>	El-Kady <i>et al.</i> , 1994; Abdel-Mallek <i>et al.</i> , 1993

TLC plates when developed in certain solvent systems (Ling *et al.*, 1979). The early reports on aflatoxin production by *Penicillia* and *Aspergilli* outside section *Flavi* were rejected by Bösenberg and Becker (1972), Frank (1972), Hesseltine *et al.* (1966), Langone and van Vunakis (1976), Mislivec *et al.* (1968), Parrish *et al.* (1966), Rabie and Terblanche (1967), Rehm (1972), Scott (1965) and Wilson *et al.* (1968). One of the first reports to show that *Aspergillus oryzae* was able to produce aflatoxin was published by El-Hag and Morse (1976). However, the culture of *A. oryzae* they used was shown to be contaminated by an aflatoxin producing

*A. parasiticus* (Fennell, 1976). Despite the fact that this problem was solved, later others repeatedly reported that *A. oryzae* was able to produce aflatoxins. Floccose strains of *A. flavus* and *A. nomius* may superficially look like *A. oryzae*, so this macromorphological resemblance may have been the reason for later erroneous reports of aflatoxin production by this species. Since *A. oryzae* is a domesticated form of *A. flavus*, the former species will not be isolated from natural sources, except if they escape the soy sauce production plants and similar factories and contaminate

Table 2. Other species incorrectly reported to produce aflatoxins.

Species	Reference
Zygomycota	
<i>Absidia butleri</i> , <i>Absidia glauca</i>	Swelim <i>et al.</i> , 1994
<i>Cunninghamella echinulata</i>	Swelim <i>et al.</i> , 1994
<i>Mucor</i> sp.	Hanssen, 1969; Sodhi <i>et al.</i> , 1985
<i>Mucor circinelloides</i> , <i>M. griseocyaneus</i> , <i>M. mucedo</i>	Swelim <i>et al.</i> , 1994
<i>Rhizopus</i> sp.	Kulik and Holaday, 1966; Van Walbeek <i>et al.</i> , 1968
<i>Rhizopus nigricans</i>	Swelim <i>et al.</i> , 1994
<i>Syncephalastrum racemosum</i>	Swelim <i>et al.</i> , 1994
Ascomycota	
<i>Alternaria cheiranthi</i>	Swelim <i>et al.</i> , 1994
<i>Cephalosporium curticeps</i> , <i>C. rosea-griseum</i>	Swelim <i>et al.</i> , 1994
<i>Cladosporium cladosporioides</i> , <i>C. sphaerospermum</i>	Swelim <i>et al.</i> , 1994
<i>Penicillium</i> sp.	Schneider <i>et al.</i> , 1972; Lee <i>et al.</i> , 1975; Sodhi <i>et al.</i> , 1985; Kulkarni <i>et al.</i> , 1986; Kraemer and Stussi, 1998
<i>P. baamense</i>	Janicki <i>et al.</i> , 1972
<i>P. brevicompactum</i>	Janicki <i>et al.</i> , 1972
<i>P. chrysogenum</i>	Swelim <i>et al.</i> , 1994; Janicki <i>et al.</i> , 1972
<i>P. citrinum</i>	Kulik and Holaday, 1966; De Waart <i>et al.</i> , 1975
<i>P. cyaneum</i>	Janicki <i>et al.</i> , 1972
<i>P. cyclopium</i>	Janicki <i>et al.</i> , 1972
<i>P. digitatum</i>	Hanssen and Jung, 1973
<i>P. expansum</i>	Hanssen and Jung, 1973
<i>P. frequentans</i>	Kulik and Holaday, 1966; De Waart <i>et al.</i> , 1975
<i>P. funiculosum</i>	Swelim <i>et al.</i> , 2001; Janicki <i>et al.</i> , 1972
<i>P. glaucum</i>	Hanssen and Jung, 1973
<i>P. oxalicum</i>	Swelim <i>et al.</i> , 1994
<i>P. puberulum</i>	Hodges <i>et al.</i> , 1966; De Waart <i>et al.</i> , 1975
<i>P. raistrickii</i>	Janicki <i>et al.</i> , 1972
<i>P. roquefortii</i>	Swelim <i>et al.</i> , 1994
<i>P. variable</i>	Kulik and Holaday, 1966; De Waart <i>et al.</i> , 1975
<i>P. verrucosum</i>	Ahmed <i>et al.</i> , 2005
<i>P. wortmannii</i>	Janicki <i>et al.</i> , 1972
" <i>P. citromyces strictum</i> "	Kulik and Holaday, 1966
<i>Scopulariopsis brevicaulis</i>	Swelim <i>et al.</i> , 1994
Bacteria	
<i>Streptomyces</i> sp.	Mishra and Murthy, 1968
Actinomycetes	Koul, 1987

the immediate surroundings. A detailed account on this issue is given by Jørgensen (2007).

Although sterigmatocystin is an intermediate of aflatoxin biosynthesis (Frisvad, 1989), only *A. ochraceoroseus* (Frisvad *et al.*, 1999; Klich *et al.*, 2000), and some *Emericella* species accumulate both sterigmatocystin and aflatoxin (Frisvad *et al.*, 2004; Frisvad and Samson, 2004). Members of *Aspergillus* section *Flavi* (*Aspergillus flavus* species group according to Raper and Fennell, 1965) which includes the major aflatoxin producers, efficiently convert sterigmatocystin into 3-methoxysterigmatocystin and then into aflatoxins (Frisvad *et al.*, 1999, 2004). The major source

of sterigmatocystin in foods is *A. versicolor*. This fungus is common on cheese, but may also occur on other substrates (Pitt and Hocking, 1997). In addition, sterigmatocystin is also produced by a high number of other *Aspergillus* species; it has been reported from species in sections *Flavi*, *Aspergillus*, *Nidulantes*, *Usti*, *Terrei* and *Flavipedes*. The production of sterigmatocystin has been confirmed in the following *Aspergillus* and *Emericella* species: *A. versicolor*, *A. rambellii*, *A. ochraceoroseus*, *E. acristata*, *E. astellata*, *E. aurantiobrunnea*, *E. bicolor*, *E. cleistominuta*, *E. corrugata*, *E. dentata*, *E. discophora*, *E. echinulata*, *E. foeniculicola*, *E. foveolata*, *E. fructiculosa*, *E. heterothallica*, *E. lata*, *E. navahoensis*, *E. nidulans*, *E. olivicola*, *E. quadrilineata*,



*E. rugulosa*, *E. spectabilis*, *E. striata*, *E. varicolor*, and *E. venezuelensis* (Ballantine *et al.*, 1965; Chexal *et al.*, 1975; Frisvad, 1985; Holzapfel *et al.*, 1966; Rabie *et al.*, 1977; Horie *et al.*, 1979, 1989; Horie and Yamazaki, 1985; Yamazaki *et al.*, 1980; Zalar *et al.*, 2008), and a fungus identified as *A. multicolor* (Hamasaki *et al.*, 1980). Another group of Aspergilli in section *Usti*, *A. ustus* and *A. puniceus*, are able to produce austocystins (Steyn and Vlegaar, 1974) and compounds related to sterigmatocystin, while *A. granulatus* can also produce a sterigmatocystin-related extrolite (Houbraken *et al.*, 2007).

Sterigmatocystin production could not be confirmed in other Aspergilli reported previously to produce this compound, for example, in *Emericella unguis* (Barnes *et al.*, 1994; Mislivec *et al.*, 1975), in *A. egyptiacus* (Moubasher *et al.*, 1977), in *Eurotium rubrum* (*E. herbariorum*), *E. repens*, *E. chevalieri*, *E. pseudoglaucom* and *E. amstelodami* (trace amounts) (Abramson *et al.*, 1983; Ahmed *et al.*, 2005; Bukelskiene *et al.*, 2006; El-Kady *et al.*, 1994; Karo and Hadlok, 1982; Labuda and Tancinova, 2006; Sanchis *et al.*, 1982; Schroeder and Kelton, 1975; Szebiotko *et al.*, 1981), *A. sydowii* and *A. aureolatus* (Abdel-Mallek *et al.*, 1993), *A. japonicus* (Begum and Samajpati, 2000) or *Aspergillus togoensis* = *Stilbothamnium togoense* (Wicklow *et al.*, 1989). Production of sterigmatocystin by *Penicillium* species has not been reported, apart from an obscure reference to *Penicillium luteum* in Dean (1963). However, Wilson *et al.* (2002) claimed that *P. camemberti*, *P. commune* and *P. griseofulvum* produce sterigmatocystin. Perhaps they have mistaken sterigmatocystin for cyclopiazonic acid. However, sterigmatocystin production also occurs in the phylogenetically unrelated genera *Monocillium* (Ayer *et al.*, 1981), *Chaetomium* (Barnes *et al.*, 1994; Koyama *et al.*, 1991; Sekita *et al.*, 1981; Udagawa *et al.*, 1979a,b), *Humicola* (Joshi *et al.*, 2002) and *Bipolaris* species (Maes and Steyn, 1984; Rabie *et al.*, 1976). As the strains of *Farrowia* and *Achaetomiella* (Holzapfel *et al.*, 1966) reported to produce sterigmatocystin are regarded as belonging to *Chaetomium* (Cannon, 1986; Udagawa, 1980) sterigmatocystin production may have evolved only once in *Chaetomium*, but this is unlikely since at least eight species have been reported to produce sterigmatocystin in *Chaetomium* so far: *C. caprinum*, *C. cellulolyticum*, *C. gracile*, *C. longicolleum*, *C. tetraspermum*, *C. thielavioideum*, *C. udagawae* and *C. virescens*.

Another precursor of aflatoxins, norsolorinic acid has also been incorrectly claimed to be produced by *A. niger* and *A. ochraceus* (Reddy *et al.*, 2005).

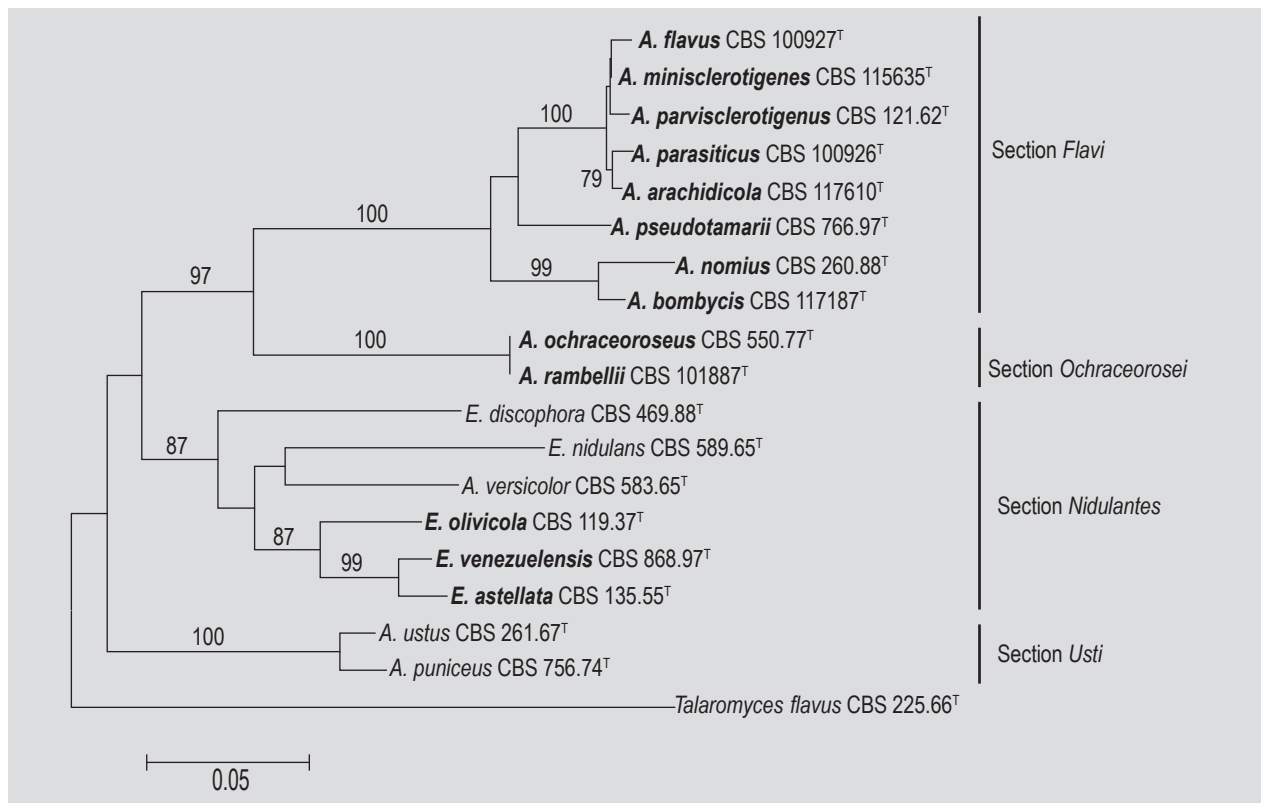
Apart from the different types of aflatoxins and sterigmatocystin another fungal metabolite related to aflatoxins, dothistromin, has also been identified (Bradshaw *et al.*, 2002). This metabolite is produced by *Dothistroma septosporum* (= *D. pini* = *Scirrha pini*)

(Baer *et al.*, 1970), *Cercospora arachidicola* (Stoessl, 1984), *C. ferruginea*, *C. fusca*, *C. microsora*, *C. rosicola*, *C. rubi*, *C. smilacis*, other *Cercospora* species (Assante *et al.*, 1977a,b), and *Mycosphaerella laricina* (Stoessl *et al.*, 1990), all pathogens belonging in the ascomycete order Dothideales. *D. septosporum* causes red-band needle blight in a wide range of pine species, a disease that leads to needle death, premature defoliation and, in severe cases, tree death (Bradshaw and Zhang, 2006). In contrast to the situation in aflatoxin-producing fungi where 25 aflatoxin biosynthetic and regulatory genes are tightly clustered in one region of the genome, the dothistromin gene cluster of *D. septosporum* is fragmented. Three mini-clusters of dothistromin genes have been identified, each located on a 1.3 Mb chromosome and each grouped with non-dothistromin genes (Zhang *et al.*, 2007). The aflatoxin precursors averufin and averyrthrin were isolated from *Cercospora smilacis* together with dothistromin (Danks and Hodges, 1974; Assante *et al.*, 1977b; Stoessl, 1984).

Recent data indicate that all known aflatoxin producing species belong to three sections of the *Aspergillus* genus: sections *Flavi*, *Ochraceorosei* and *Nidulantes*. Among these, sections *Nidulantes* and *Ochraceorosei* are assigned to subgenus *Nidulantes*, while section *Flavi* belongs to subgenus *Circumdati* based on multilocus sequence based phylogenetic studies (Peterson *et al.*, 2008). A tree based on phylogenetic analysis of  $\beta$ -tubulin sequence data depicting relationships of aflatoxin- and some of the sterigmatocystin-producing Aspergilli is shown in Figure 2.

### ***Aspergillus* section *Flavi***

*Aspergillus* section *Flavi* historically includes species with conidial heads in shades of yellow-green to brown, and dark sclerotia. Isolates of the so-called domesticated species, such as *A. oryzae*, *A. sojae* and *A. tamarii* are used in oriental food fermentation processes and as hosts for heterologous gene expression (Campbell-Platt and Cook, 1989). The economically most important aflatoxin producers belong to this section of the *Aspergillus* genus. Aflatoxins have been shown to be produced by *A. flavus*, *A. parasiticus* (Codner *et al.*, 1963; Schroeder, 1966), *A. nomius* (Kurtzman *et al.*, 1987), *A. pseudotamarii* (Ito *et al.*, 2001), *A. bombycis* (Peterson *et al.*, 2001), *A. toxicarius* (Murakami, 1971; Murakami *et al.*, 1982), *A. parvisclerotigenus* (Saito and Tsureta, 1993; Frisvad *et al.*, 2005), *A. zhaoqingensis* (Sun and Qi, 1991), *A. flavus* var. *columnaris* (Van Walbeek *et al.*, 1968), *A. minisclerotigenes* and *A. arachidicola* (Pildain *et al.*, 2008). Aflatoxin B<sub>2</sub> was found as a minor extrolite in all aflatoxin B<sub>1</sub> producing species, but as the only type of aflatoxin in *A. flavus* var. *columnaris* NRRL 5821 and IBT 12654 and in *A. zhaoqingensis* CBS 399.93. *A. zhaoqingensis* produced kojic acid, aspergillilic acid, one aflatoxin (B<sub>2</sub>), and tenuazonic acid like most strains of *A. nomius* (unpublished data). Molecular data indicate that *A. flavus* var. *columnaris*



**Figure 2. Phylogenetic tree of aflatoxin and sterigmatocystin-producing *Aspergillus* species based on  $\beta$ -tubulin sequence data. Aflatoxin producers are set in bold type. Numbers above branches indicate bootstrap values; only values above 70% are indicated.**

and *A. zhaoqingensis* are synonyms of *A. flavus* and *A. nomius*, respectively (Pildain *et al.*, 2008; data not shown). Apart from aflatoxins B and G, aflatoxin M<sub>1</sub> and M<sub>2</sub> have also been found to be produced by some *A. flavus* and *A. parasiticus* isolates (Ramachandra *et al.*, 1975; Engel, 1978; Dutton *et al.*, 1985; Pallavi *et al.*, 1997; Lopez-Diaz *et al.*, 1996).

*Aspergillus flavus* is the most common species producing aflatoxins (Sargeant *et al.*, 1961) and other economically important mycotoxins including e.g. cyclopiazonic acid (Dorner *et al.*, 1983) and gliotoxin (Fox and Howlett, 2008), occurring in most kinds of foods in tropical countries. This species is very common on maize, peanuts and cottonseed, and produces only B-type aflatoxins. It has been estimated that only about 30-40% of known isolates produce aflatoxin. *A. flavus* populations are genetically and phenotypically diverse (Geiser *et al.*, 2000) with some isolates producing conidia abundantly, produce large (L) sclerotia, and variable amounts of aflatoxins, while another type produces abundant, small (S) sclerotia, fewer conidia and high levels of aflatoxins (Cotty, 1989). Probst *et al.* (2007) found that the 2004 aflatoxicosis outbreak in Kenya was caused by S-type isolates of *A. flavus* which produced large amounts of aflatoxin B<sub>1</sub>. A related species, *A. oryzae* is atoxigenic and has been used as a source of industrial enzymes and as a koji (starter) mould for Asian fermented

foods, such as sake, miso, and soy sauce (Van den Broek *et al.*, 2001). *A. oryzae* isolates carry various mutations in the aflatoxin biosynthetic gene cluster resulting in their inability to produce aflatoxins (Tominaga *et al.*, 2006). Notably, the *aflR* gene is absent or significantly different in some *A. oryzae* strains compared to *A. flavus* (Lee *et al.*, 2006).

There were many reports indicating that certain *A. flavus* strains including micro-sclerotial strains, and strains listed as intermediate between *A. flavus* and *A. parasiticus* can also produce type G aflatoxins (Begum and Samajpati, 2000; Codner *et al.*, 1963; Cotty and Cardwell, 1999; Hesseltine *et al.*, 1970). Recent molecular investigations of these isolates revealed that they belong to two separate species, *A. parvisclerotigenus* and *A. minisclerotigenes* (Frisvad *et al.*, 2005; Pildain *et al.*, 2008). Many other isolates producing both aflatoxins B and G and bearing small sclerotia have been reported to date (Bayman and Cotty, 1993; Cotty and Cardwell, 1999; Egel *et al.*, 1994; Frisvad *et al.*, 2005; Saito and Tsuruta, 1993). Further studies are necessary to assign these isolates to species.

*Aspergillus pseudotamarii* (Ito *et al.*, 2001) is another effective producer of B type aflatoxins but its importance as regards mycotoxin occurrence in foods is unknown. The closely related species *A. tamarii* is not able to produce

aflatoxins, despite several reports claiming this (Goto *et al.*, 1996; Klich *et al.*, 2000).

Aflatoxins G<sub>1</sub> and G<sub>2</sub> are found in *A. parasiticus*, *A. nomius*, *A. bombycis*, *A. parvisclerotigenus*, *A. minisclerotigenes*, *A. arachidicola* and *A. toxicarius*. *Aspergillus parasiticus* occurs rather commonly in peanuts, but is apparently quite rare in other foods. It is more restricted geographically than *A. flavus*. *A. parasiticus* produces both B and G aflatoxins (Sargeant *et al.*, 1963), and virtually all known isolates are toxigenic. This species also produces kojic acid and aspergillic acid. *A. toxicarius*, another species closely related to *A. parasiticus* also produces B- and G-type aflatoxins (Murakami *et al.*, 1966; Murakami, 1971). Sequence analysis of multiple loci indicate that *A. toxicarius* is a synonym of *A. parasiticus* (Pildain *et al.*, 2008). *A. sojae* is the domesticated variety of *A. parasiticus*, which can scarcely be distinguished from it except for its inability to produce aflatoxins (Chang *et al.*, 2007; Rigó *et al.*, 2002). The lack of aflatoxin-producing ability of some *A. sojae* isolates results primarily from an early termination point mutation in the pathway-specific *AflR* regulatory gene, which causes the truncation of the transcriptional activation domain of *AflR* and the abolition of interaction between *AflR* and the *AflJ* co-activator. In addition, a defect in the polyketide synthase gene also contributes to its inability to produce aflatoxins (Chang *et al.*, 2007).

*Aspergillus nomius* and *A. bombycis* are two related species also producing both aflatoxins B and G, whereas neither produces cyclopiazonic acid (Peterson *et al.*, 2001; Table 3). *A. bombycis* was isolated from silkworm-rearing houses in Japan and Indonesia, whereas *A. nomius* is more widespread: it was originally isolated from mouldy wheat in the USA, and later from various substrates in India, Japan and Thailand. Peterson *et al.* (2001) observed cryptic recombination in *A. nomius* populations using multilocus sequence data. Recently, Olsen *et al.* (2008) have observed that *A. nomius* is an important producer of aflatoxins in Brazil nuts.

*A. minisclerotigenes* was isolated from Argentinean peanuts with small sclerotia and produces aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, aspergillic acid, cyclopiazonic acid, kojic acid, parasiticolides and several other extrolites (Pildain *et al.*, 2008; Table 3). One of the strains listed by Hesseltine *et al.* (1970), NRRL A-11611 = NRRL 6444 also produced aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, aflatrem, aflavinines, aspergillic acid, cyclopiazonic acid, parasiticolides, kojic acid, paspaline, paspalinine and emindole SB and is conspecific with *A. minisclerotigenes*. *A. minisclerotigenes* also includes isolates assigned by Geiser *et al.* (2000) to *A. flavus* group II. Another species producing small sclerotia and both aflatoxins B and G is *A. parvisclerotigenus* (Frisvad *et al.*, 2005). The type strain of this species (CBS 121.62 = NRRL A-11612 = IBT 3651 = IBT 3851) was isolated from peanuts (*Arachis hypogea*) in Nigeria, and has an extrolite profile very similar to that of

*A. minisclerotigenes*, but in contrast with the Argentinean strains, it also produces parasiticolides, and compound A 30461 (aspirochlorin = oryzaechlorin; Table 3).

*A. arachidicola* was isolated from leaves of *Arachis glabrata* in Argentina, and produces aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, aspergillic acid, chrysogine, oryzaechlorin, parasiticolide, and extrolites NO<sub>2</sub> and EPIF. All strains had a floccose colony texture, a conidium colour similar to *A. flavus* but, except for the production of chrysogine by most isolates, they exhibited extrolite profiles similar to those of *A. parasiticus* isolates (Pildain *et al.*, 2008; Table 3).

Members of *Aspergillus* section *Flavi* that produce aflatoxin B<sub>1</sub> also produce kojic acid, and, except for *A. bombycis* and *A. pseudotamarii*, aspergillic acid (Table 3). 3-O-methyl-sterigmatocystin was found in all aflatoxin producers. Species examined that produced the G type aflatoxins usually do not produce cyclopiazonic acid and vice versa as suggested by Takahashi *et al.* (2004). However, some isolates of two recently identified aflatoxigenic species, *A. parvisclerotigenus* and *A. minisclerotigenes* are able to produce both extrolites in culture (Frisvad *et al.*, 2005; Pildain *et al.*, 2008). Members of section *Flavi* produce different combinations of aflatoxins, kojic acid, cyclopiazonic acid and aspergillic acid and only share the aflatoxins (B type) with species in the sections *Ochraceorosei* and *Nidulantes*. Furthermore, none of the strains that produced aflatoxins in *Aspergillus* section *Flavi* produced detectable amounts of sterigmatocystin, while sterigmatocystin was always accumulated together with aflatoxin B<sub>1</sub> in aflatoxigenic *Emericella* species and in *A. ochraceoroseus* and *A. rambellii* (Frisvad *et al.*, 2005).

### ***Aspergillus* section *Ochraceorosei***

This section was established by Frisvad *et al.* (2005), and includes species not able to grow at 37°C, producing yellow ellipsoidal conidia, biserial conidial heads and long, smooth conidiophore stipes. This section includes two aflatoxigenic species, *A. ochraceoroseus* and *A. rambellii*, both isolated from soil in Ivory Coast. These are the only species known to accumulate aflatoxin B<sub>1</sub> and sterigmatocystin simultaneously. 3-O-methylsterigmatocystin was also detected in these cultures. *A. ochraceoroseus* has previously been assigned either to *Aspergillus* sections *Wentii*, *Cremeri* or *Circumdati* (Christensen, 1982; Kozakiewicz, 1989; Samson, 1979). However, phylogenetic analysis of sequence data from aflatoxin and sterigmatocystin genes *aflR* and *nor-1/stcE*, as well as ITS and  $\beta$ -tubulin genes of aflatoxin producing species indicated that *A. ochraceoroseus* was related more closely to the species in subgenus *Nidulantes* than to species from subgenus *Circumdati* (Cary *et al.*, 2005; Klich *et al.*, 2003). *A. ochraceoroseus* produced more aflatoxin B<sub>1</sub> than *E. venezuelensis* and *E. stellata*, but less than members of section *Flavi*, whereas *A. rambellii*

**Table 3. Morphological characteristics, occurrence and extrolites of aflatoxin producing species (modified after Frisvad et al., 2006).**

Section	Morphological characteristics	Occurrence	Extrolites produced	Reference
<i>Flavi</i>				
<i>A. bombycis</i>	mostly biseriatae, sclerotia not reported	Japan, Indonesia	aflatoxins B & G, kojic acid, aspergillilic acid	Peterson et al., 2001
<i>A. flavus</i>	mostly biseriatae, sclerotia large or small	Worldwide	aflatoxins B <sub>1</sub> & B <sub>2</sub> , kojic acid, cyclopiazonic acid, aspergillilic acid, asperfuran, paspalinine, paspaline	Codner et al., 1963
<i>A. nomius</i>	mostly biseriatae, sclerotia small, bullet-shaped	USA, Thailand, Japan, India, Brazil	aflatoxins B & G, kojic acid, aspergillilic acid, tenuazonic acid, nominine	Kurtzmann et al., 1987
<i>A. parasiticus</i>	mostly uniseriate, sclerotia uncommon, large	USA, Japan, Australia, India, South America, Uganda	aflatoxins B & G, kojic acid, aspergillilic acid, paspalinine, paspaline	Schroeder, 1966
<i>A. parvisclerotigenus</i>	mostly biseriatae, sclerotia small, spherical	Nigeria	aflatoxins B & G, parasiticol, cyclopiazonic acid, kojic acid, 3-O-methylsterigmatocystin, versicolorins, a and b afatrem, paspalinine, paspaline, aflavarin, aspirochlorin	Frisvad et al., 2005
<i>A. minisclerotigenes</i>	mostly biseriatae, sclerotia small, spherical	Argentina, USA, Australia, Nigeria	aflatoxins B & G, kojic acid, aspergillilic acid, paspalinine, paspaline, cyclopiazonic acid, aflavarins, paspalinin, paspaline, aflatrem, aflavinines	Pildain et al., 2008
<i>A. arachidicola</i>	mostly biseriatae, sclerotia small, spherical	Argentina	aflatoxins B & G, kojic acid, aspergillilic acid, parasiticolides, chrysogine	Pildain et al., 2008
<i>A. pseudotamarii</i>	biseriate, sclerotia large, spherical	Japan, Argentina	aflatoxin B <sub>1</sub> , kojic acid, cyclopiazonic acid	Ito et al., 2001
<i>Ochraceorosei</i>				
<i>A. ochraceoroseus</i>	biseriate, sclerotia absent	Ivory Coast	aflatoxins B <sub>1</sub> & B <sub>2</sub> , sterigmatocystin, 3-O-methylsterigmatocystin, kotanin-like metabolites, wortmannin-like metabolites, indole alkaloids	Frisvad et al., 2005
<i>A. rambellii</i>	biseriate, sclerotia absent	Ivory Coast	aflatoxins B <sub>1</sub> & B <sub>2</sub> , sterigmatocystin, 3-O-methylsterigmatocystin, versicolorins, averufin, norsolorinic acid, kotanin/desertorin-, wortmannin-, emerlin/xanthocillin- and indole-like extrolites	Frisvad et al., 2005
<i>Nidulantes</i>				
<i>E. astellata</i>	biseriate, ascomata and Hülle-cells, ascospores stellate	Ecuador	aflatoxin B <sub>1</sub> , arugosin A & B, asperthecin, shamixanthone, sterigmatocystin, terrein, variecoxanthone A, B & C	Frisvad et al., 2004
<i>E. olivicola</i>	biseriate, ascomata and Hülle-cells, ascospores stellate	Italy	aflatoxin B <sub>1</sub> , arugosin E, siderin, shamixanthone, sterigmatocystin, terrein, varitriols	Zalar et al., 2008
<i>E. venezuelensis</i>	biseriate, ascomata and Hülle-cells, ascospores stellate	Venezuela	aflatoxin B <sub>1</sub> , sterigmatocystin, terrein, compounds with chromophores of the shamixanthone, emerlin and desertorin type	Frisvad and Samson, 2004



produces the greatest amounts of aflatoxin B<sub>1</sub> ever observed, even more than the best producers in section *Flavi* (*A. parasiticus*, *A. parvisclerotigenus* and *A. nomius*; Frisvad *et al.*, 2005).

### **Aspergillus section *Nidulantes***

*Aspergillus* section *Nidulantes* (*A. nidulans* species group) includes some species which are able to reproduce only asexually, and the anamorphs of *Emericella* species. Characteristics of this section include the production of conidial heads that are typically short columnar, and in the shades of dark yellow-green to bluish green, and Hülle cells that are usually globose to citriform in shape. Raper and Fennell (1965) assigned 18 species to this section. Samson (1979) added 10 more species. *Emericella* is a genus containing species of considerable interest because of the well elucidated genetics of *E. nidulans* and because some species produce penicillin (Dulaney, 1947a,b). Several other species of *Emericella* produce sterigmatocystin (El Khady and Abdel Hafez, 1981; Frisvad *et al.*, 2004), while three species, *E. astellata* (Frisvad *et al.*, 2004), *E. venezuelensis* (Frisvad and Samson, 2004) and *E. olivicola* (Zalar *et al.*, 2008) have been found to be able to produce aflatoxins in this section. *Emericella astellata* and *E. venezuelensis* produce the typical short brown to yellow brown conidiophores and small vesicles covered with metulae with phialides in their upper part. *E. astellata* and *E. venezuelensis* grow rather slowly or not at all at 37 °C (0 and 0-9 mm after one week of incubation, respectively) in contrast to most other species of *Emericella*. *E. astellata* produces aflatoxin B<sub>1</sub>, arugosins, asperthecin, shamixanthone, sterigmatocystin, terrein and varicoxanthones, while *E. venezuelensis* produces aflatoxin B<sub>1</sub>, sterigmatocystin, terrein, and compounds with chromophores of the shamixanthone, emerlin and desertorin type (Frisvad *et al.*, 2004; Frisvad and Samson, 2004). Another aflatoxin producing *Emericella* species, *E. olivicola* grows well at 37 °C, and has stellate ascospores with relatively narrow equatorial crests (up to 2.3 µm). The thin-walled Hülle cells are filled with oil droplets (Zalar *et al.*, 2008). This species produces numerous extrolites including arugosin E, siderin, shamixanthone, sterigmatocystin, terrein, varitriols, and aflatoxin B<sub>1</sub>, of which the latter was detected only in one of the two strains. In *Emericella* species in general, aflatoxins were always minor components compared to other extrolites.

### **3. Conclusions**

In this review a current overview of aflatoxin-producing species is provided. Since their discovery of these mycotoxins in the early sixties, several *Aspergilli* and other fungi have been claimed to be able to produce aflatoxins. However, most of these reports were incorrect. To date, aflatoxin producing abilities of 14 *Aspergillus* species have been confirmed, 3 of which belong to section *Nidulantes*, 2

to section *Ochraceorosei* (both sections belong to subgenus *Nidulantes*; Peterson, 2008), while the remaining 11 species are assigned to section *Flavi*. Based on these findings, aflatoxin biosynthesis thus seems to have evolved at least twice within the *Aspergillus* genus. The situation is more complex in the case of sterigmatocystin, which has been found to be produced by several *Aspergilli* assigned to sections *Nidulantes*, *Flavi* and *Ochraceorosei*, but also by species in the phylogenetically unrelated genera *Monocillium*, *Chaetomium*, *Humicola* and *Bipolaris*. Production of sterigmatocystin may have evolved independently two or three times in *Aspergillus* and its teleomorphs, and at least four times in the unrelated genera listed above.

Regarding the economical importance of aflatoxin producing species, *A. flavus* and *A. parasiticus* are the most frequently encountered aflatoxigenic species in various food products including peanuts, maize, cotton and spices. Recent data indicate that *A. nomius* may contribute to aflatoxin contamination of Brazil nuts (Olsen *et al.*, 2008), and the significance of the newly described microsclerotial species, *A. minisclerotigenes* and *A. arachidicola*, needs further investigations. The remaining aflatoxin-producing species are less significant from the point of view of aflatoxin contamination of foods and feeds, since they are rare (e.g. *A. ochraceoroseus* or *E. venezuelensis*), have a restricted distribution (e.g. *A. bombycis* was predominately isolated from silkworm rearing houses), or – to the best of our knowledge - do not contaminate foods (e.g. *E. astellata* or *A. pseudotamarii*).

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