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# Black aspergilli and ochratoxin A-producing species in foods

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Ochratoxin A (OTA) is a potent nephrotoxin and carcinogen which is found in a wide variety of common foods and beverages. The black aspergilli are distributed worldwide and are regarded as common food spoilage fungi. These fungi are one of the more difficult groups concerning classification and identification. New molecular approaches have shown that there is a high biodiversity, but that species are occasionally difficult to recognize based solely on their phenotypic characters. Only few species have been confirmed to be OTA producers in this group and fewer are known to contaminate foods with this mycotoxin as a natural occurring contaminant. In this paper, the OTA-producing species included in the *Aspergillus* section *Nigri* and the foods that they are able to contaminate are reviewed in depth.

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## Introduction

Ochratoxin A (OTA) is a potent nephrotoxin and carcinogen which is found in a wide variety of common foods and beverages [1]. The International Agency for Research on Cancer classified OTA as a possible human carcinogen (group 2B). Cereals are considered the major source of human exposure to OTA. For this reason many countries have set a limit for OTA in cereals [2]. However, human exposure to OTA is most likely coming from low level contamination of a wide range of different foods. In order to prevent or minimize this risk, the European Union have established maximum OTA levels for many foodstuffs such as cereals, coffee, dried vine fruit, liquorice, spices, wheat gluten and wine [3]. Nonetheless, this regulatory status of OTA lacks consensus in other countries [4].

More than a half century has elapsed since OTA was discovered as a metabolite of *Aspergillus ochraceus* [5••]. A few years later, *Penicillium verrucosum* (as *Penicillium viridicatum*) was reported to produce also this mycotoxin [6•,7]. Traditionally, OTA contamination of food and feeds was believed to be restricted to *A. ochraceus* and *P. verrucosum*, which affect mainly dried stored foods and cereals respectively, in different regions of the world [8]. Currently, the list of OTA-producing species has expanded and, consequently, the list of foods which can be contaminated with this mycotoxin has also increased. However, although a high number of additional *Aspergillus* species are able to produce OTA, few of them are known to contaminate foods with this mycotoxin [9].

Of these latter species, those include in the *Aspergillus* section *Nigri* (black aspergilli) are becoming of interest because some of them are able to colonize foods other than cereals and cereal by-products and to contaminate them with OTA. In this paper, the OTA-producing species included in this section and the foods that they are able to contaminate are reviewed in depth.

## The black aspergilli (*Aspergillus* section *Nigri*)

The black aspergilli are distributed worldwide and are regarded as common food spoilage fungi. Some species are widely used in the biotechnolgy industry and a few of them are involved in animal or human mycoses. These fungi are one of the more difficult groups concerning classification and identification, and several taxonomic schemes have been proposed [10•]. In the last revision of the genus *Aspergillus*, 27 species were accepted in the *Aspergillus* section *Nigri* [11••]. Basic information about these taxa based on the last taxonomic proposals [11••,12,13,14•,15] are summarized in Table 1. Some of these species are rare or recently described, and only a few strains of them have been studied. Consequently, the distribution of these species it is not well known.

The main morphological character of the most common species (e.g. *A. niger*) included in this section is the color of the conidial head, which is black or some shade of black. For this reason, these fungi form generally characteristic black or dark brown colonies (Figure 1). However, the color of the colonies varies not only in different species, but in the same strain, depending upon the culture medium, the age of the culture, the abundance of sporulation or the production of sclerotia, among other factors. Consequently, depending on these factors, some species

**Table 1**

**Current accepted species in the *Aspergillus* section *Nigri*, their main ecological characteristics and OTA production**

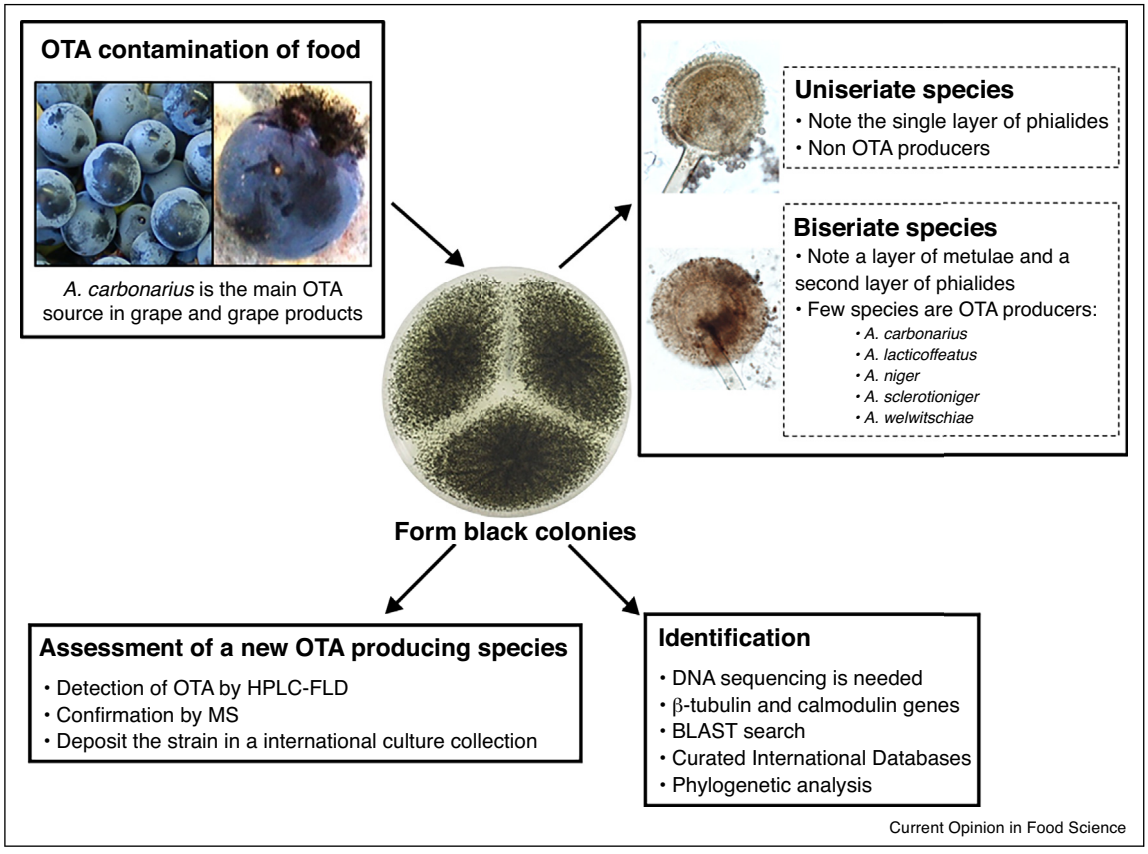
| Species, authorities and year of the description | Molecular clade | Conidial head | Ecology, main habitats and distribution | OTA production[a]/ OTA source[b] |
|---|---|---|---|---|
| *A. aculeatinus*, Noonim *et al.*, 2008 | *A. aculeatus* | Uniseriate | Coffee beans, Thailand | – |
| *A. aculeatus*, Iizuka, 1953 | *A. aculeatus* | Uniseriate | Tropical soil, unknown, air, USA, *Lactuca sativa*, Indonesia, dead branches, Papua | – |
| *A. brasiliensis*, Varga *et al*., 2007 | *A. niger* aggregate | Biseriate | Soil, wine grapes, worldwide | – |
| *A. brunneoviolaceus*, Batista & Maia, 1957 | *A. aculeatus* | Uniseriate | Air, Trinidad and Tobago, and USA | – |
| *A. carbonarius*, (Bainier) Thom, 1916 | *A. carbonarius* | Biseriate | Soil, wine grapes, coffee, figs, maize, paprika, peanuts, worldwide | +/Wine, dried vine fruits and other grape products (cocoa, coffee)[b] |
| *A. costaricaensis*, Samson & Frisvad, 2004 | *A. niger* aggregate | Biseriate | Soil, Costa Rica | – |
| *A. ellipticus*, Raper & Fennell, 1965 | *A. heteromorphus* | Biseriate | Soil, Costa Rica | – |
| *A. eucalypticola*, Varga *et al*., 2011 | *A. niger* aggregate | Biseriate | Leaves of *Eucalyptus* sp., Australia | – |
| *A. floridensis*, Jurjević *et al*., 2012 | *A. aculeatus* | Uniseriate | Air, USA and Martinique, soil, Japan, almonds USA | – |
| *A. heteromorphus*, Batista & Maia, 1957 | *A. heteromorphus* | Biseriate | Fungal culture contaminant, Brazil | – |
| *A. homomorphus*, (Steiman *et al*.) Samson & Frisvad, 2004 | *A. homomorphus* | Biseriate | Soil of death sea area, Israel | – |
| *A. ibericus*, Serra *et al*., 2006 | *A. carbonarius* | Biseriate | Wine grapes and raisins, Portugal and Spain | – |
| *A. indologenus*, Frisvad *et al*., 2011 | *A. aculeatus* | Uniseriate | Soil, India | – |
| *A. japonicus*, Saito, 1906 | *A. aculeatus* | Uniseriate | Soil, Brazil | – |
| *A. labruscus*, Fungaro *et al*., 2017 | *A. homomorphus* | Uniseriate | Grapes for juice production, Brazil | – |
| *A. lacticoffeatus*, Frisvad & Samson, 2004 | *A. niger* aggregate | Biseriate | Coffee beans, Indonesia and Venezuela | +/(coffee)[b] |
| *A. luchuensis*, Inui, 1901 | *A. niger* aggregate | Biseriate | East Asia, food fermentation environment | – |
| *A. neoniger*, Varga *et al.*, 2011 | *A. niger* aggregate | Biseriate | Mangrove water, Venezuela, and desert sand, Namibia | – |
| *A. niger*, van Tieghem, 1867 | *A. niger* aggregate | Biseriate | Worldwide, cosmopolitan fungus | +/(many foods)[b] |
| *A. piperis*, Samson & Frisvad, 2004 | *A. niger* aggregate | Biseriate | Grounded black pepper of tropical origin | – |
| *A. saccharolyticus*, Sørensen *et al*., 2011 | *A. homomorphus* | Uniseriate | Oak wood, Denmark | – |
| *A. sclerotiicarbonarius*, Noonim *et al.*, 2008 | *A. carbonarius* | Biseriate | Coffee beans, Thailand | – |
| *A. sclerotioniger*, Samson & Frisvad, 2004 | *A. carbonarius* | Biseriate | Green Arabica coffee, India | +/(coffee)[b] |
| *A. trinidadensis*, Jurjević *et al.*, 2012 | *A. aculeatus* | Uniseriate | Air, Trinidad and Tobago, and USA | – |
| *A. tubingensis*, Mosseray, 1934 | *A. niger* aggregate | Biseriate | Worldwide, cosmopolitan fungus | – |
| *A. uvarum*, Perrone *et al*., 2008 | *A. aculeatus* | Uniseriate | Wine grapes, Europe and Israel, air, USA | – |
| *A. vadensis*, Samson *et al.*, 2005 | *A. niger* aggregate | Biseriate | Air, Egypt | – |
| *A. welwitschiae*, (Bresadola) Hennings apud Wehmer, 1907 | *A. niger* aggregate | Biseriate | Grapes, dried fruits, coffee, cocoa, worldwide | +/(many foods)[b] |

[a] OTA production: +, OTA producing species; –, non-OTA producing species.
[b] (Potential source).
Data are from Cabañes and Bragulat [9], Samson *et al.* [11••], Varga *et al.* [12], Jurjević *et al.* [13], Hong *et al.* [14•], Fungaro *et al.* [15], Samson *et al.* [18], Taniwaki *et al.* [40], Copetti et al. [56•].

can form purple-brown, reddish-brown, dark greenish, yellow or orange colonies [16•]. In the conidial head, some species have a single layer of phialides on the vesicle and are named uniseriate species (Table 1 and Figure 1). However, most of them have biseriate conidiophores (Figure 1), showing a layer of metulae on the vesicle and a second layer of phialides over them. These are named biseriate species (Table 1).

New molecular approaches have shown that there is a high biodiversity in this group. However, these species are difficult to recognize based solely on their phenotypic characters [16•]. For example, the taxa related to *A. niger* have always been extremely difficult to distinguish by morphological means. In these fungi, the differences between the described species and varieties in the proposed classifications are very subtle and the number of taxa varies from one author to another [10•]. For this group of fungi, it was proposed the *A. niger* aggregate. The term aggregate is used in mycology for groups of closely related morphospecies only distinguishable with difficultly. Nowadays, this group comprise ten species [11••], including *A. brasiliensis*, *A. niger* and *A. tubingensis* which are the most common species isolated from foods. Although there is a substantial call for searching for new molecular and physiological markers that are usable in the

**Figure 1**




Importance, detection and identification of black aspergilli in foods. (This figure summarizes the main concepts discussed).

classification of black aspergilli, the concept of several recently described species was only based on genetic differences at one locus [17].

Nevertheless, new polyphasic taxonomic approaches have been proposed for the classification and identification of these fungi [11••,16•]. These polyphasic studies include sequence analysis of some genes such as ITS-5.8S rRNA region (ITS), and β-tubulin (BenA) and calmodulin (CaM) genes, morphological analyses and characterization of extrolite profiles, among other characters. Although ITS is considered the universal DNA barcode of fungi, these sequences do not contain enough variation for distinguishing among all species in the *Aspergillus* section *Nigri*. A secondary barcode or identification marker usually is needed to identify a black aspergilli culture to species level with confidence [11••]. For example, the uncommon species *A. lacticoffeatus* is characterized by its hair brown to dark blonde colonies which is an important distinguishing feature in its description [18] (Figure 2). However, *A. lacticoffeatus* is very close molecularly to *A. niger* sensu stricto [18]. In fact, this species has been considered a color mutant of *A. niger* [12]. They have identical ITS sequences and can not be separated by their BenA sequences but can be distinguished using CaM sequence data and have also a different extrolite profile [16•]. This new taxonomic approach has also allowed to determine that some black aspergilli which are used in the production of Asian fermented foods and beverages, such as *A. luchuensis*, *A. coreanus*, *A. kawachii* and *A. acidus* were the same species. *A. luchuensis* was selected as the correct name based on priority [11••,12,14•].

In the last 10 years, following these taxonomical approaches, the number of described uniseriate species has dramatically increased from two to eleven species (see Table 1). Only, *A. aculeatus* and *A. japonicus* were recognized previously [10•,18]. Consequently, nowadays, in order to accurately identify black aspergilli, in addition to characterize the micromorphology (e.g. conidial head, conidia) of an isolate is necessary to compare ITS and some protein-coding gene sequences such as BenA and CaM with published sequences in curated International Databases [11••,19••]. The use of CaM as a temporary secondary identification marker in *Aspergillus* has been suggested [11••]. Using these markers, these taxa can be

**Figure 2**


Colonial morphology of *A. lacticoffeatus* (CBS 101883), grown on Czapek yeast extract agar at 30°C for 10 days. Note the distinctive coffee-with-milk color of the colony.

divided into five main molecular clades (e.g. *A. aculeatus*, *A. carbonarius*, *A. heteromorphus*, *A. homomorphus* and *A. niger* aggregate clades) (see Table 1).

## OTA producing species in the black aspergilli

Only a few black aspergilli have been confirmed to be OTA producers and fewer are known to contaminate foods with this mycotoxin as a natural occurring contaminant. In the biseriate species, only five species are considered to be able to produce OTA. In the *A. niger* aggregate, the phylogenetically close species *A. niger* and *A. welwitschiae* are considered OTA producers (Figure 3). They are black aspergilli morphologically indistinguishable.

The common species *A. niger* has a worldwide distribution and the reported percentage of OTA-producing strains in this species is usually very low [10•]. It is one the most commonly reported species from foods (e.g. sun dried products, fresh fruits, nuts, cereals) [1]. Nevertheless, since the first description of OTA production by *A. niger* [20•] this species is achieving a greater significance regarding OTA content in some food commodities such as grapes, raisins and wine, and also in coffee. Interestingly, this species is perhaps the most important mold used in biotechnology. It is worth noting that *A. niger* products hold the GRAS (Generally Regarded as Safe) status from the Food and Drug Administration and is a widely applied industrial species for large-scale biotechnological production of organic acids and enzymes in the food industry. However, some of the most frequently used strains in industry were able to produce this toxin on media suggested for citric acid production [21•]. Among other things, due to their potential for mycotoxin production, no filamentous fungi has the QPS (Qualified Presumption of Safety) status proposed by the European Food Safety Authority [22•].

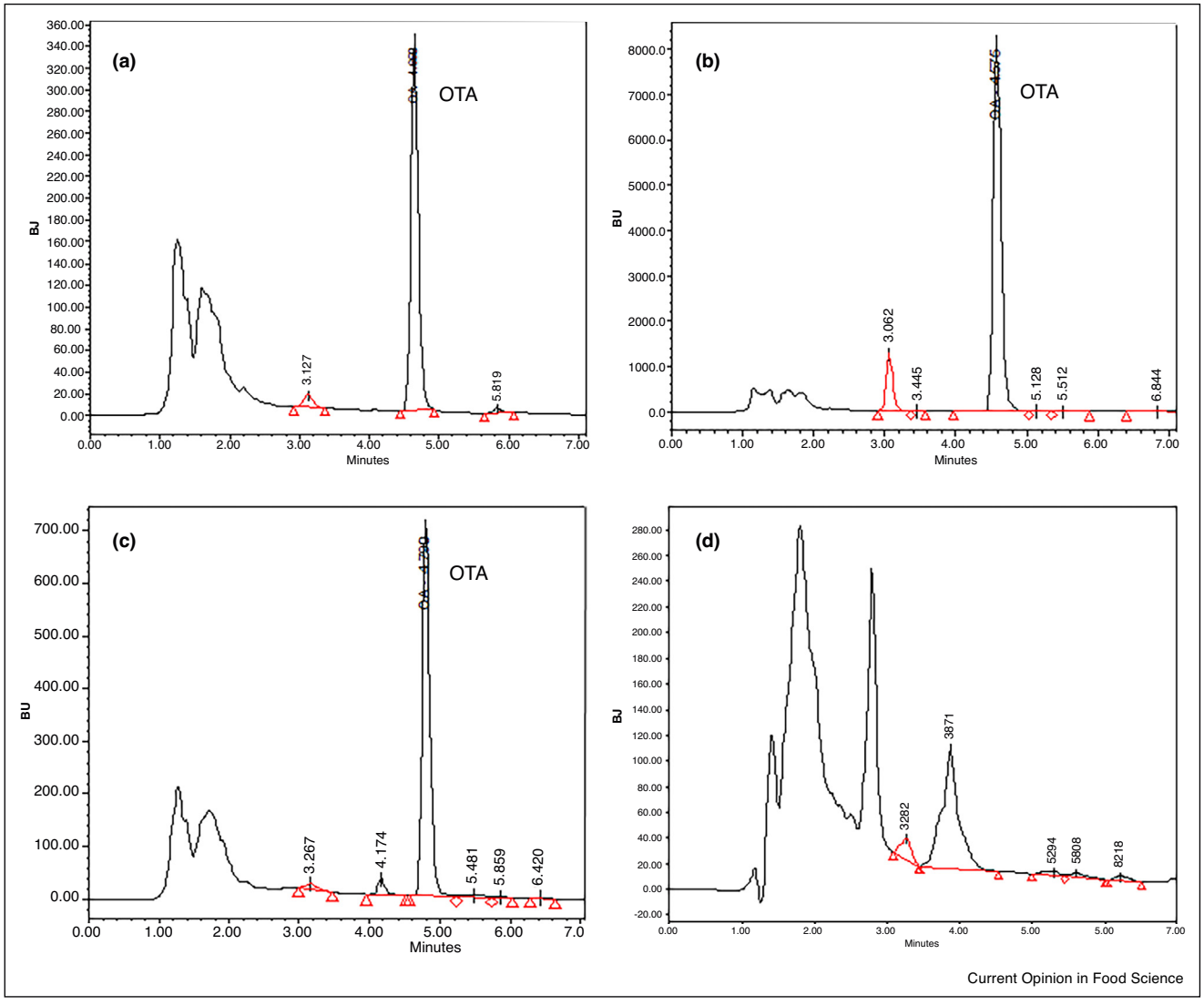
Regarding *A. welwitschiae*, which was previously called *A. niger* or *A. niger* var. *phoenicis*, most strains produce occasionally OTA. This species has not yet been reported frequently because this old species name has been reintroduced in a recent study about the elucidation of the taxonomic position of some black aspergilli involved in the awamori fermentation [14•]. Consequently, *A. welwitschiae* is expected to be more frequently reported in a near future. It has been found on Welwitschia plants, grapes, dried fruits, coffee, cocoa, and other sources and it has also a worldwide distribution from all the continents [14•].

On the other hand, the rare species *A. lacticoffeatus* which is located in the same molecular subclade as *A. niger* and *A. welwitschiae* (*A. niger*/*welwitschiae* subclade) [11••], is also able to produce OTA over a wide range of temperatures [18,23] (Figure 3). However, in this case, *A. lacticoffeatus* which was isolated from coffee beans, forms distinctive coffee-with-milk colored colonies (Figure 2).

Outside this group, *A. carbonarius* is very consistent in producing this mycotoxin, and non-OTA-producing strains in this species are very rare [9,24,25•] (Figure 3). In some cases, the suspected non-OTA producing isolates previously identified as *A. carbonarius* belonged actually to other different black aspergilli species [26,27]. The conidia of *A. carbonarius* are much larger (>6 μm) than those of most of the species in section *Nigri*. A large number of studies have shown that *A. carbonarius* is the main responsible source of OTA in wine or dried vine fruits from main viticultural regions worldwide [28•,29–39]. It is also considered a potential source of OTA in coffee. This species has been reported from coffee beans from various coffee-producing countries such as Brazil [40], Philippines [41], Thailand [42,43] and Vietnam [44]. However, *A. ochraceus* and *A. westerdijkiae* (section *Circumdati*) are considered the most significant source of OTA contamination in some coffee varieties [40,43]. Although it is not so common as *A. niger*, *A. carbonarius* has been also reported from other foods such as figs, maize, paprika, peanuts among others [1].

On the other hand, *A. sclerotioniger* which is phylogenetically close to *A. carbonarius*, has been also confirmed as an OTA producer. This species has also large conidia and produce sclerotia. Nevertheless, *A. sclerotioniger* is known from only one strain (CBS 115572) which was isolated from green Arabica coffee beans in India [18].

**Figure 3**




Selected chromatograms of fungal extracts analyzed using HPLC coupled to a fluorescence detector of **(a)** an OTA-producing strain of *A. carbonarius*, **(b)** an OTA-producing strain of *A. niger*, **(c)** an OTA-producing strain of *A. lacticoffeatus* and **(d)** a non-OTA-producing strain of *A. tubingensis*.

Natural occurrence of OTA in maize and maize-based products is a worldwide problem. The available information on the ochratoxigenic mycobiota and OTA presence in corn, corn based food and feed is limited. Several surveys have been shown that *A. niger* and *A. ochraceus* could be the main source of OTA [45]. It is well known that *P. verrucosum* is the major producer of OTA in cereals such as wheat and barley in temperate and cold climates, and, although *A. ochraceus* has been isolated from a wide range of cereals, records are rather infrequent [46,47]. However, *A. niger* is frequently isolated from maize [1,48–50] and a high incidence of *A. carbonarius* has been also reported in this product [49]. It has been speculated that both species could be a source of OTA in maize and other food products in both tropical and subtropical zones of the world [51]. In fact, both species were able to produce OTA in maize kernels from the fifth day of incubation over a wide range of temperatures and water availabilities [52].

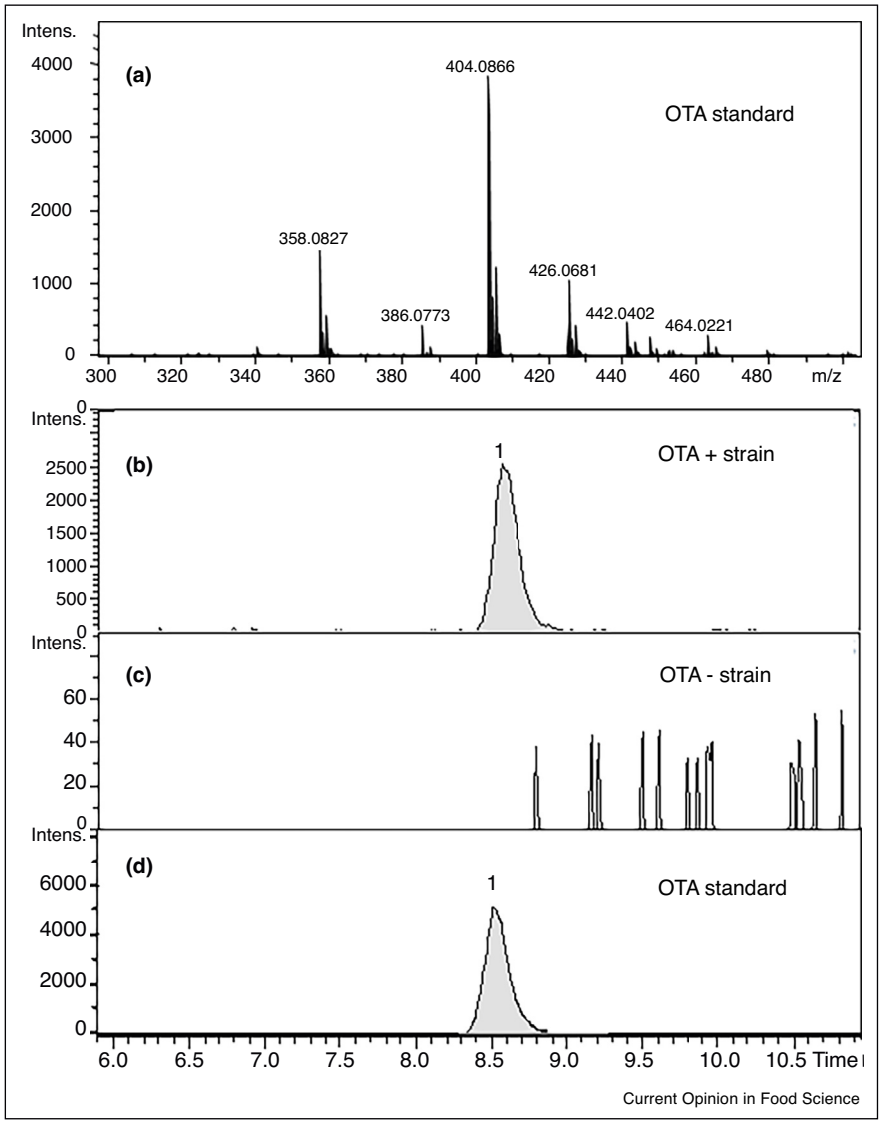
OTA-producing strains of both species have been also reported from cocoa beans. Several studies have shown that *A. carbonarius* and *A. niger* are important fungal sources of OTA in cocoa [53–55]. However, ochratoxigenic strains of *Aspergillus melleus*, *A. westerdijkiae* and *A. ochraceus* (section *Circumdati*) have also been reported [55]. Different molds may contaminate many stages in cocoa processing, and poor practices may have a strong

influence on the quality of the beans. OTA is found at all stages of cocoa processing, with the major incidence during drying and storage. In fact, the contamination of cocoa by OTA-producing fungi can already take place in the fermentation, but a considerable increase in the numbers of these species, as well in ochratoxin A contamination is observed during drying and storage [55,56•]. More systematic surveys on different stages of cocoa production in other important cocoa producing countries are needed in order to confirm the OTA fungal sources in these products.

In addition to cereals, coffee, dried vine fruit and wine, the European Union have established maximum OTA levels also on other foodstuffs such as liquorice and spices, including dried spices, *Piper* spp. (including white and black pepper), *Myristica fragrans* (nutmeg), *Zingiber officinale* (ginger), *Curcuma longa* (turmeric), *Capsicum* spp. (including chillies, chilli powder, cayenne and paprika) and mixtures of spices containing one of the above mentioned spices [3].

At present, the source of OTA in liquorice is not known. In recent studies in China [57,58], some *Penicillium* spp., such as *P. polonicum* and *P. chrysogenum*, among other fungal species have been reported to be a possible source of OTA in this product. These authors claimed that *P. chrysogenum* derived from surrounding environments was

**Figure 4**




Electrospray ionization–mass spectrometry spectrum of OTA **(a)** (major ions: *m/z* 358.08 [MH-HCOOH]+, *m/z* 404.09 [MH]+ and *m/z* 426.07 [MNa]+) and selected extracted ion chromatograms of fungal extracts of an OTA-producing strain of *A. carbonarius* **(b)** and a non-OTA-producing strain of *A. carbonarius* **(c)** analyzed using HPLC-MS. OTA standard **(d)**.

likely to be a stable contributor to high OTA level in liquorice. However, none of these species are considered OTA producers [47,59]. This should be confirmed using proper chromatographic detection of OTA and accurate identification of the fungi.

Currently, the source of OTA in spices is also unknown. However, *A. niger* has been reported as the most frequently isolated species in *Piper* spp. [60,61], *Myristica fragrans* [62], *Zingiber officinale* [61], *Curcuma longa* [61] and *Capsicum* spp. [61,63–65]. Some other potential OTA-producing species such as *A. ochraceus* and *A. westerdijkiae* (section *Circumdati*) were also reported in some of these studies, but less frequently. Interestingly, solid-state fermentation with *A. niger* is used in the process of pepper peeling. So, some OTA-producing starter strains of this species could be the OTA source in this product. This kind of fermentation is widely used in traditional Chinese food fermentation due to its easy operation, low cost, and wide feasibility on farms and in the countryside [60].

Some strains of other black aspergilli such as *A. awamori*, *A. usamii* and *A. foetidus* have been also cited as OTA producers [10•,66]. However, their identity has been questioned [14•,18]. Most *A. awamori* strains isolated from oriental food fermentation process could be accommodated into *A. niger* group, such as *A. luchuensis*, *A. niger*, *A. tubingensis* or *A. welwitschiae*. In fact, the neotype of *A. awamori* (CBS 557.65) did not originate from awamori fermentation and it was shown to be identical with *A. welwitschiae* [14•].

The ability of *A. tubingensis* to produce OTA remains a controversial issue. Some strains of this species have been cited as OTA producers [67–69]. However, *A. tubingensis* is not considered an OTA producer [10•,18,21•] (Figure 3). In fact, a high number of strains of this species tested by other authors were not able to produce OTA [28•,29,37,70–74]. Very recently, none of the 261 *A. tubingensis* strains isolated from wine grapes was found to be ochratoxigenic when they were analyzed with UPLC-MS/MS [74]. Nevertheless, some of these isolates were initially considered to be able to produce the toxin when they were screened by HPLC-FLD. Consequently, OTA production by strains of *A. tubingensis* and their taxonomical identity, should be confirmed using proper techniques. Similarly, none of the uniseriate species are considered to be able to produce OTA [66,71,73,75–77]. Although the ability of some of these species to produce OTA has been mentioned [30,78–80], this fact needs to be confirmed.

As an unusual OTA-producing species is proposed, an accurate identification of the isolate and a proper detection of the OTA production must be carried out in different culture media and conditions in order to confirm that is a new OTA fungal source (Figure 1). OTA confirmation by mass spectrometry is recommended (Figure 4). In order to avoid the chronic problem of misidentification of mycotoxigenic fungi some useful recommendations have been proposed [9,72,81].

## Conclusions

*Aspergillus* section *Nigri* is one of the more difficult fungal groups concerning classification and identification. Only a few of these fungi are able to produce OTA. Some of these OTA-producing species can contaminate a wide variety of foods and beverages. Nowadays, we know that *A. carbonarius* is the main responsible source of OTA in wine or dried vine fruits from main viticultural regions worldwide. Although there is clear evidence of the participation of *A. carbonarius* and *A. niger* on the OTA contamination of cocoa and coffee, their exactly role have not been stated. On the other hand, it is not always clear which black aspergilli species are responsible for OTA contamination in other foods. While *A. niger* and *A. welwitschiae* are usually reported from a wide variety of foods, their role as a source of OTA is not well known. In fact, *A. niger* is frequently isolated from some EU regulated foods such as liquorice and spices. Nevertheless, the source of OTA in these foods has not been identified yet. More systematic research is needed to confirm which black aspergilli species are responsible for the OTA contamination in these and other commodities.

## Conflict of interest

None declared.

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- • of special interest
- •• of outstanding interest

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