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Aflatoxins in sugarcane production chain: what could be the source?

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Reports on the occurrence of aflatoxin producing fungi and aflatoxins in the sugarcane value chain (juice, milled sugarcane, stalk, jaggery and dried yeast samples) have been published in some countries. *Aspergillus parasiticus* was identified as the main species isolated from the sugarcane system. However, with the introduction of polyphasic taxonomy, new species have been described in the *Aspergillus* section *Flavi* and a recent report revealed that *Aspergillus novoparasiticus* and *Aspergillus arachidicola* (to a lesser extent) were the main species isolated in samples throughout the sugarcane processing chain. This review aims to highlight the main reports of aflatoxigenic species and aflatoxins in sugarcane and discuss the significance of these species on its production chain.

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Introduction

Sugarcane (*Saccharum officinarum* L.) is a perennial grass belonging to the family Poaceae, cultivated mainly in tropical and subtropical climate regions. Sugarcane production represents a significant economic element in several countries, especially those with a primary commodity-based economy. By 2017, the area harvested was about 26 million hectares. The world's largest sugarcane producers are Brazil, India and China [1].

Sugarcane contributes to 80% of global sugar production. In addition, in Brazil, this plant is directly used to produce ethanol on a large scale. The by-products from sugarcane processing, namely the straw and bagasse (cane fibres), can be used to produce cellulosic ethanol, a second-generation biofuel. Other sugarcane products include molasses, rum, and cachaça (a Brazilian drink), and the plant itself can be used as thatch and as livestock fodder.

Recently, *Aspergillus novoparasiticus* and *Aspergillus arachidicola* (to a lesser extent), both producing aflatoxins, were found in the sugarcane processing chain samples, such as: milled sugarcane, stalk, soil, and dried yeast [2•]. These species are readily known to produce aflatoxins of type B and G [3,4]. Aflatoxins are one of the main classes of mycotoxins in foods, possessing hepatotoxic, immunosuppressive, and carcinogenic activity [5]. In developing countries, many people are exposed to aflatoxins through home-grown food, where inadequate production and storage techniques favour the proliferation of aflatoxin-producing fungi. An estimated 4.5 billion people living in developing countries may be chronically exposed to aflatoxins through their diet [6]. Apparently, *A. novoparasiticus* seems to be the main species in the contamination of sugarcane by aflatoxins. This review aims to reverberate the main reports of *A. novoparasiticus* in sugarcane and to cover the role of this species in the value chain of this important commodity.

The sugarcane value chain: energy, food and byproducts

Nowadays, compliance with renewable energies is almost mandatory to maintain good business relations globally. In the last few decades the use of vegetal biomass for the production of liquid fuels has been intensified, for example, in Brazil about 70% of the renewable energy produced is of bioenergetic matrix [7], with most of this due to the production of ethanol, obtained through the fermentation of sugar by yeasts, followed by the distillation process. Ethanol production is being driven by the technology of hybrid vehicles, coupled with the search for cleaner energy sources. Processing of sugarcane to obtain sugar and ethanol involves different stages and some by-products are generated. Among them, dried yeast stands out. Yeast in its viable form is used to ferment the juice extracted from sugarcane and the 'wine' produced is distilled to obtain alcohol. One part of the yeast is recovered to be used in a new fermentation step and about 10% is removed from the process to be dried. This part is purified and used as a protein source for feed and food, due to its high protein and amino acid content, about 30–60% [8,9]. Because of the expansion of the

production of sugarcane and alcohol, there is a tendency to increase the dried yeast production to be used as a protein source. According to Aquarone *et al.* [10] about 1.5 kg of dried yeast per 100 L of produced alcohol was generated, based on Brazilian alcohol production data in 2018/2019. It is estimated that 495 M t of dry yeast were produced in this period.

Sugarcane juice can be concentrated to produce crystalline sucrose which is purified to obtain different types of sugar. The fibrous residue of the plant (bagasse) produced from this process can be burned in boiler systems to provide heat and steam in the pre-processing industries of the sugar-alcohol chain.

In villages, chewing of raw sugarcane is a common practice, whereas in cities, freshly extracted sugarcane juice is a popular drink and sold by the local vendors. Sugarcane juice, sweet in taste, provides an instant source of energy and calories. It has a low glycemic index, being healthier than table sugar, and keeps the body hydrated. Besides, sugarcane juice is also rich in phenolic acids, flavonoids, and antioxidant compounds [11,12]. Jaggery is a natural sweetener made by concentrating the sugarcane juice without adding any chemicals [13]. It contains up to 50% sucrose, 20% invert sugars, vitamins, and minerals with some other insoluble matter such as ash, proteins, and bagasse fibers [14,15]. Active ingredients such as iron and vitamin C present in jaggery can weaken the genotoxic effects caused by arsenic [16]. Antioxidant phenolic compounds in jaggery were reported as cytoprotective against tetra-butyl hydroperoxide and hydrogen peroxide induced oxidative damage [17].

Aflatoxins and their producers in sugarcane and its byproducts

The occurrence of aflatoxigenic fungi and aflatoxins in sugarcane and its byproducts has been reported by some authors [2•,18,19]. Aflatoxins are a family of fungal secondary metabolites produced by some filamentous fungi and are a cause of great concern in both animal and human health because of their clear relationship with hepatic cancers, with aflatoxin B_1 being the main mycotoxin [20].

In a study carried out in India, among the 57 sugarcane juice samples collected from local markets, 22.2% and 19% of sugarcane juice from Mysore and Mandya were contaminated with aflatoxins, respectively. The levels of contamination ranged from 0.5 to 6.5 μg/kg. Moreover, from 71 jaggery samples, 4.8% (Mysore) and 6.6% (Mandya) recorded aflatoxin contamination ranging from 0.5 to 1.0 μg/kg [19].

Iamanaka *et al.* [2•] investigated aflatoxin contamination throughout the sugarcane chain and reported that most samples of sugarcane juice (68.5%), molasses (100%), cream yeast (86.4%) and dried yeast (73%) showed contamination by this class of toxin, the levels of contamination (total aflatoxins) ranged from 0.4 μg/kg to 10.2 μg/kg.

Sugarcane is a major crop in the southernmost islands of Japan. The sugarcane product of muscovado (crude sugar) is well known as a natural food and is sometimes used for confectionery or other dishes. According to Kumeda *et al.* [21•], since 1993, aflatoxin B_1 has been detected in muscovado that is produced in this area.

The main aflatoxin producing species are found in *Aspergillus* section *Flavi*. Currently, eighteen species of this group are recognized as aflatoxin producers: *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nomius*, *Aspergillus pseudonomius*, *A. novoparasiticus*, *Aspergillus pseudotamarii*, *Aspergillus togoensis*, *Aspergillus pseudocaelatus*, *Aspergillus luteovirescens*, *Aspergillus minisclerotigenes*, *A. arachidicola*, *Aspergillus sergii*, *Aspergillus transmontanensis*, *Aspergillus mottae*, *Aspergillus aflatoxiformans*, *Aspergillus austwickii*, *Aspergillus pipericola* and *Aspergillus cerealis* [4,22].

A. parasiticus has been pointed out as the main aflatoxigenic species occurring in sugarcane and byproducts. However, Iamanaka *et al.* [2•] reported *A. novoparasiticus* as the predominant species, and a substantial occurrence of *A. arachidicola* in sugarcane, but not *A. parasiticus*. This new finding is curious and raises the reassessment of the previous understanding since *A.* section *Flavi* taxonomy has been very dynamic and frequently revised. In the last decade, a total of 18 new species have been described in *A.* section *Flavi* (*A. arachidicola*, *A. minisclerotigenes*, *A. pseudocaelatus*, *A. pseudonomius*, *A. sergii*, *A. transmontanensis*, *A. mottae*, *A. cerealis*, *A. novoparasiticus*, *Aspergillus bertholletius*, *Aspergillus hancockii*, *A. aflatoxiformans*, *Aspergillus aspearensis*, *A. austwickii*, *Aspergillus neoalliaceus*, *Aspergillus subflavus*, *A. pipericola* and *Aspergillus vandermerwei)*. The last taxonomic revision of *A.* section *Flavi* was performed, in 2019, by Frisvad *et al.* [22], who recognized 33 valid species in this section. Considering the importance of correctly identifying the toxigenic species, it is necessary to review the context of *A.* section *Flavi* in sugarcane and byproducts against the taxonomic *status quo* of the group.

A. parasiticus or *A. novoparasiticus*, who's the villain in sugarcane case?

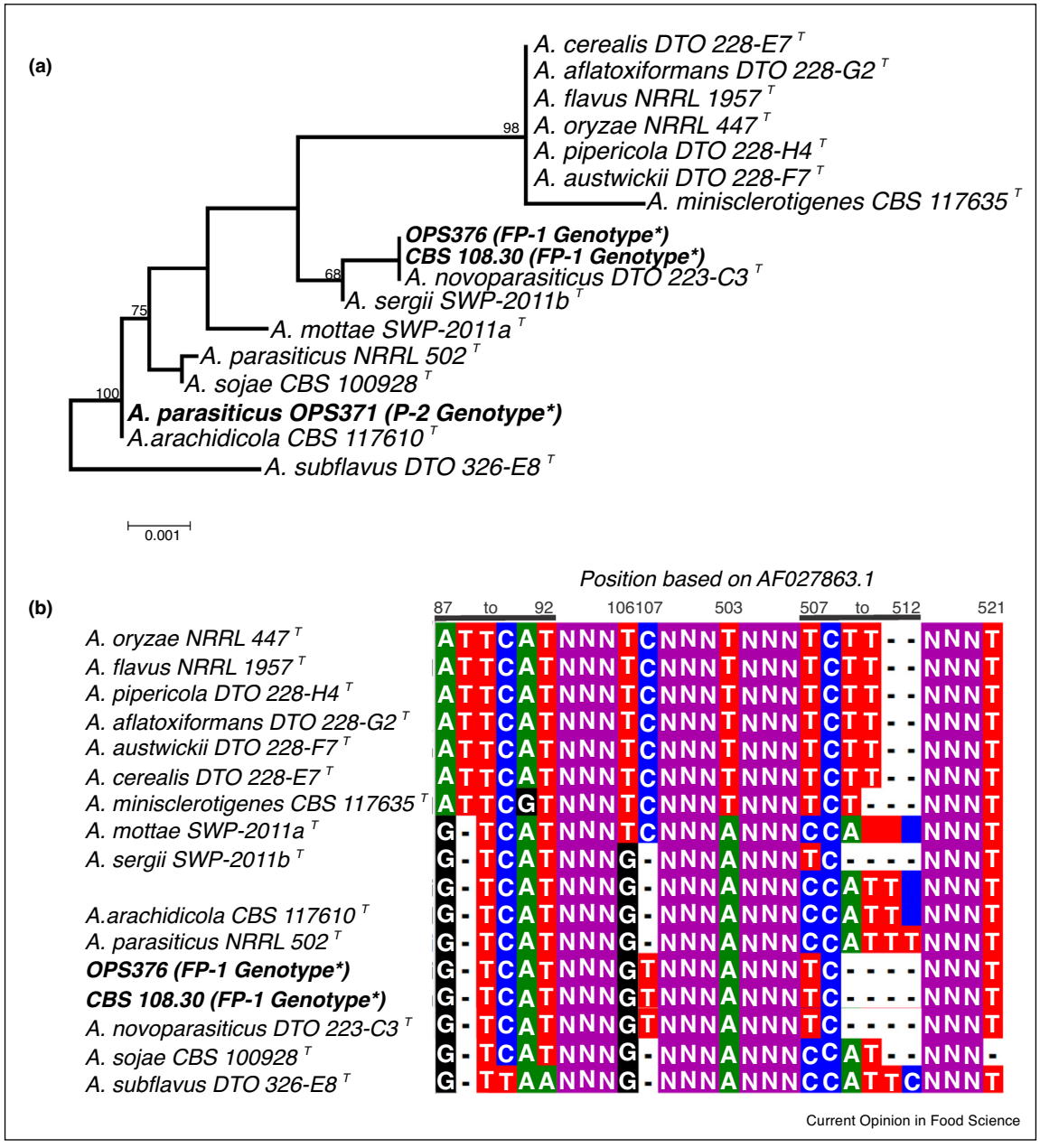
There are a few studies of the occurrence of aflatoxin producing fungi in sugarcane [2•,21•,23,24•]. All of them, except Iamanaka *et al.* [2•], reported *A. parasiticus* as the main aflatoxigenic species isolated from sugarcane and sugarcane soil ecosystems. *A. parasiticus* was not found by Iamanaka *et al.* [2•], instead, *A. novoparasiticus* was the main species isolated in most samples. Among the 226 isolates of *A.* section *Flavi* obtained from the sugarcane production chain, almost 85% were identified as *A. novoparasiticus* [2•].

A. novoparasiticus presents intermediate characteristics between *A. flavus* and *A. parasiticus* and was originally described in 2012 by Gonçalves *et al.* [3], who isolated it from clinical sources in Brazil (sputum leukemic patient). The description of this species was supported by

phylogenetic analysis (multilocus), physiology (especially toxigenic profile; aflatoxin producer B_1, B_2, G_1, and G_2), and morphology. Subsequently, this species was isolated from cassava [25], corn [26], rice [27], and yerba mate [28].

Kumeda *et al*. [21•] used Heteroduplex Panel Analysis (HPA) of the internal transcribed spacer (ITS) region of the rRNA gene to identify strains of *A*. section *Flavi*. Through this technique, the authors identified 19 HPA profiles, and at the time, these profiles were identified by the authors as *A. flavus*/*A. oryzae* (F-1 to F-6), *A. parasiticus*/*Aspergillus sojae* (P-1 and P-2), *Aspergillus tamarii* (T-1), *A. nomius* atypica (TN-1), *A. nomius* (N-1 to N-3), *A. pseudotamarii* (T-2), *Aspergillus caelatus* (T-3), *Aspergillus bombycis* (TN-2), *A. nomius* (N-4 and N-5), and a new genotype that has been reported as FP-1. All 71 strains of the 'FP-1 genotype' had been isolated from sugarcane or sugarcane field soil in the southernmost islands of Japan, Vietnam, and Egypt, and all were able to produce aflatoxins type B and G.

Figure 1



(a) Neighbor joining tree base on ITS region of *Aspergillus flavus* clade type strains species and FP-1 (OPS376 and CBS 108.30) and P-2 (OPS371) genotypes founded in sugarcane by Kumeda *et al*. [21•] (* in bold). **(b)** Box showing the differences, in the ITS sequences, between the type strains of all *A. flavus*-clade species and FP-1 and P-2 genotypes founded in sugarcane by Kumeda *et al*. [21•] (* in bold).

The FP-1 genotype included strain CBS 108.30, originally isolated from sugarcane mealybugs (*Pseudococcus sacchari*) in Egypt. Previously, Wang *et al.* [29] had already characterized this strain as a different group in *A.* section *Flavi*, based on the mitochondrial cytochrome b gene [29].

Phylogenetic analysis performed through an NJ tree, constructed on the basis of ITS sequence data, demonstrated that FP-1 strains formed a separate clade of *A. flavus* (F-1 to F-6 genotypes) and *A. parasiticus* (P-1 and P-2 genotypes) (see Figure 4 in Kumeda *et al.* [21•]). According to the authors, the FP-1 genotype consists of isolates that are morphologically intergrading between *A. parasiticus* and *A. flavus*. All this evidence led the authors to conjecture on the possibility that FP-1 genotype could represent a new species in *A.* section *Flavi*. However, these authors [21•] did not take this study further.

The ITS region is considered the official barcode for fungi [30]. Based on the ITS sequence from two representatives of the genotype FP-1 (OPS 376 = NCBI accession AB074996 and CBS 108.30 = NCBI accession AB074996), we were able to visualize the data of Kumeda *et al.* [21•] from the perspective of current taxonomic status (2019 *status quo*).

As shown in Figure 1, in fact, FP-1 genotype representatives (OPS 376 and CBS 108.30S strain) have an ITS sequence identical to type strain of *A. novoparasiticus* (LEMI 250 = CBS 126849 = DTO 223-C3 = DTO 223-C4 = FMR 10121 = IBT 32311). Although, the ITS region does not work well to identify all species in *Aspergillus* genus [31], fortunately, this region discriminates well *A. novoparasiticus* and *A. parasiticus*. In addition, the strain OPS 371 isolated from sugarcane in Vietnam denoted by the authors as profile P-2 (at the time as *A. parasiticus*) have an ITS sequence identical to type strain of *A. arachidicola* (Figure 1).

This species phylogenetically related to *A. parasiticus* was described in 2008 by Pildain *et al.* [32]. It was isolated from peanut samples (seeds and leaves) and their strains formed a well-supported phylogenetic group based on sequence data (*BenA*). Morphological and physiological differences that allowed their discrimination from the other species of *A.* section *Flavi* were also found.

The P-1 genotype isolated from several substrates (macadamia nuts, peanuts, mealy bugs, koji, soy sauce, forest soil, and bean) from different countries was indeed *A. parasiticus* (see *Erratum* to Figure 3 of Kumeda *et al.* [21•]). Interestingly, the FP-1, and P-2 strains found by Kumeda *et al.* [21•] as associated with sugarcane represent the same species found by Iamanaka *et al.* [2•], showing that *A. novoparasiticus* and *A. arachidicola* are ubiquitous species in the sugarcane chain.

Garber and Cotty [24•] studying sugarcane fields in Texas related the presence of *A. parasiticus* in soils cropped to sugarcane. The authors worked with Vegetative Compatibility Analyses and sequences of three *loci*: the ITS region, *niaD* and *aflR* genes. Phylogenetic trees of maximum parsimony and maximum likelihood were constructed based on *niaD*, *aflR*, and ITS data sequences. The *niaD* and *aflR* are not *loci* conventionally used in the taxonomy of *A.* section *Flavi*, so the sequences of these genes are not available for all species of this section. As far as we know, the ITS sequences obtained by Garber and Cotty [24•] are not available in Genbank, and, neither *niaD* and/or *aflR* sequences are available either for the type strain of *A. novoparasiticus* (LEMI 250). Consequently, this does not make it possible to compare the data obtained by these authors in relation to the current taxonomic scenario.

It is important to comment that Garber and Cotty [24•] have already observed that isolates of '*A. parasiticus*' obtained from sugarcane soil were grouped in a different clade of *A. parasiticus* obtained from other sources and, in addition, formed a group of consistent vegetative compatibility. This result may be a clue that the isolates obtained from sugarcane fields do not belong to species *A. parasiticus* (as was believed at the time), and probably belong to *A. novoparasiticus*. However, at this point we can only conjecture, for support for such an assertion would be necessary to obtain the *niaD* or *aflR* gene sequences for *A. novoparasiticus* type strain, which would allow a direct comparison with the sequences deposited by Garber and Cotty [24•] (NCBI accession numbers KC769488–KC769508 for *aflR* and KC782772–KC782791 for *niaD*).

Conclusion

The taxonomy of *A.* section *Flavi* has undergone many changes, mainly due to the introduction of polyphasic taxonomy and the contribution of molecular data. The association of *A. novoparasiticus* and *A. arachidicola* (to a lesser extent) with Brazilian sugarcane, its by-products and its agrosystem was consistently proved by Iamanaka *et al.* [2•]. In our vision, reviewing the data of previous studies based on current taxonomic status of *A.* section *Flavi*, this association may occur in sugarcane produced in other countries. *A. novoparasiticus* may also be the main aflatoxigenic species in sugarcane around the world. This new understanding gives grounds for new investigations. The perfect knowledge of the fungus–plant relationship will allow better strategies for the control of this species and the reduction of aflatoxin contamination in sugarcane and its byproducts. Naturally, this new information leads us to new questions, for example, why are *A. novoparasiticus* and sugarcane so intimately associated? This response remains obscure and needs further investigation.

Conflict of interest statement

Nothing declared.

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