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Aspergillus section *Flavi* diversity and the role of *A. novoparasiticus* in aflatoxin contamination in the sugarcane production chain

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ABSTRACT

The presence of *Aspergillus* section *Flavi* and aflatoxins in sugarcane as well as in by-products, such as molasses, sugar, yeast cream and dried yeast, collected from different fields and processing plants in São Paulo state, were investigated throughout the sugarcane production chain. A total of 246 samples was collected and analyzed and 226 isolates of *Aspergillus* section *Flavi* were isolated. *Aspergillus* section *Flavi* strains were found in sugarcane juice, milled sugarcane, stalk, soil and dried yeast samples. Among the isolates of *Aspergillus* section *Flavi* submitted to polyphasic identification ($n = 57$), *Aspergillus novoparasiticus* and *Aspergillus arachidicola* were predominantly found. A significant proportion of the isolates (84.5%) were found to have morphological and physiological characteristics of *A. novoparasiticus*. Most samples, with the exception of sugar, showed some aflatoxin contamination. The highest level was in dried yeast with an average of 2.55 μg/kg and maximum value of 10.19 μg/kg. This is the first report of contamination of sugarcane by *A. novoparasiticus*.

1. Introduction

Sugarcane is one of the most important agricultural products for the Brazilian economy. Production reached 657 M tonnes in 2016/2017 and the estimated production in 2017/2018 is 648 M tonnes (CONAB, 2017). Around 63% of sugarcane production is located in the South East of Brazil, mainly in São Paulo state, which is responsible for around 60% of the total Brazilian production. This high sugarcane cultivation is to maintain both ethanol and sugar production. Sugar is one of the main exported products from Brazil and is obtained by the concentration of the sugarcane juice. Ethanol is 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. In 2016/2017 the production of sugar and ethanol reached 38.7 M tonnes and 27.8 B litres, respectively (CONAB, 2017).

Processing of sugarcane to obtain these two products involves different technologies with by-products being generated. Among these, dried yeast is particularly important. This yeast in its viable form is used to ferment the juice extracted from sugarcane; the "wine" produced is distilled to obtain alcohol. A portion of this yeast is recovered for use in a new fermentation step and about 10% is removed from the process to be dried. This portion is purified and used as a protein source for feed and food, due to its high protein and amino acid content, which can be between 30 and 60% (Halász and Lásztity, 1991; Meurer et al., 2000). Due to 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. (2001) about 1.5 kg of dried yeast per 100 L of produced alcohol was generated, resulting in a total estimate production of 420 M tonnes of dried yeast production annually.

However, the increase in production in some cases leads to a lack of control of hygienic standards and possible microbiological contamination. In São Paulo and Mato Grosso states, the major sugarcane producer areas, most of the sugarcane harvesting is done mechanically, removed from the soil and accumulated in large trucks until delivery to processing companies. In this phase, sugar cane is susceptible to microbial contamination, being rich in carbohydrates with a high water

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activity (a_w), pH and optimum temperature (tropical climate). The high sugar content of these substrates can allow several fungal species to appear both in terms of visible colonization and mycotoxin contamination. The accumulation of sugar leads to a decrease in a_w (Pitt and Hocking, 2009) that together with temperature, are the main environmental parameters controlling fungal development and production of secondary metabolites, including mycotoxins (Magan and Aldred, 2007). The key genus, which is able to grow in high sugar content commodities, are the *Aspergillus* species, which includes the section *Flavi* group which can result in aflatoxin contamination.

Aflatoxins are a family of fungal secondary metabolites produced by some filamentous fungi and are 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 (IARC, 1993). Several species of *Aspergillus* section *Flavi* can produce aflatoxins including *Aspergillus flavus*, *A. parasiticus*, *A. nomius*, *A. pseudonomius*, *A. pseudocaelatus*, *A. novoparasiticus*, *A. luteovirescens* (*A. bombycis*), *A. minisclerotigenes*, *A. arachidicola*, *A. mottae*, *A. sergii*, *A. transmontanensis*, *A. pseudotamarii*, *A. togoensis*, *A. korhogoensis*, *A. aflatoxiformans*, *Aspergillus austwickii* and *A. cerealis* (Carvajal-Campos et al., 2017; Frisvad et al., 2019; Soares et al., 2012; Varga et al., 2011; Varga et al., 2015).

Important aflatoxigenic species such as *A. flavus* (18%) and *A. parasiticus* (65%) were found in sugarcane or soil in sugarcane plots in Japan (Kumeda et al., 2003; Takahashi et al., 2004). Garber and Cotty (2014) studying sugarcane fields in Texas, USA related the presence of *A. parasiticus* in soils cropped to sugarcane. However, no studies have attempted to correlate the presence of aflatoxin-producing fungi and the presence of aflatoxins in sugarcane and its by-products, in the ethanol and sugar processing chain.

Because of the increased production of sugarcane in Brazil, and the level of by-products being generated during processing there has been a need for a more detailed study. Thus, the objective of this study was to examine the presence of *Aspergillus* section *Flavi* and aflatoxins in sugarcane and its by-products including molasses, non-refined sugar, cream yeast and dried yeast, collected from different fields and processing plants in São Paulo State, were investigated throughout the sugarcane production chain.

2. Material and methods

2.1. Samples

A total of 246 samples was collected from three sugar cane processing plants in São Paulo state, including sugarcane stalks ($n = 10$), milled sugarcane ($n = 30$), sugarcane juice ($n = 73$), molasses ($n = 24$), non-refined sugar ($n = 24$), cream yeast ($n = 23$), dried yeast ($n = 54$) and soil from sugarcane plantations ($n = 8$).

Around 10 kg of sugarcane stalk were sampled from a delivery truck, using an automatic sampler, then cut and pressed to obtain approximately 500 mL of sugarcane juice. Approximately 200 mL of sugarcane juice and 200 g of milled sugarcane were collected. Molasses, non-refined sugar and cream yeast were collected during a days' production to have different lots, and around 500 g were taken during the process. Fifty-four samples of dried yeast (around 300 g) were obtained in São Paulo state. Soil (300 g) was collected in the sugarcane plantations around the plants visited.

2.2. Fungal isolation

Samples were analyzed by a serial dilution technique using peptone water (0.1%) and plated onto Dichloran Rose Bengal agar (DRBC) for isolations from sugarcane juice, milled sugarcane, molasses and cream yeast and Dichloran 18% Glycerol agar (DG18) for dried yeast, non-refined sugar and soil, according to Pitt and Hocking (2009). The plates were incubated at 25 °C for 5 days. All *Aspergillus Flavi* strains were isolated and purified on Czapek Yeast Extract Agar (CYA) and incubated at 25 °C for 5 days.

Table 1
Occurrence of *Aspergillus* section *Flavi* in sugarcane (juice, milled and stalk), molasses, unrefined sugar, cream yeast and dried yeast.

Samples (*n*)	Frequency of occurrence (%)	*Aspergillus* section *Flavi* (CFU/g or mL)		
		Average	Median	Range
Stalk (10)	50	330	50	< 100–1.0×10^3
Sugarcane juice (73)	70	6.1×10^3	1.1×10^3	< 10–1×10^5
Milled sugarcane (30)	10	3.4×10^3	< 100	< 100–1.0×10^5
Soil (8)	50	400	100	< 100–1.0×10^3
Molasses (24)	0	< 10	< 10	–
Unrefined sugar (24)	0	< 100	< 100	–
Cream yeast (23)	0	< 10	< 10	–
Dried yeast (54)	1.8	2.70	< 100	< 100–100

CFU: colony forming unit.

2.3. Morphological and physiological analysis

The *Aspergillus* section *Flavi* strains were isolated on a standard medium Czapek Yeast Extract Agar (CYA) and Malt Extract Agar (MEA), incubated at 25 °C, and also at 37 °C and 42 °C on CYA and incubated for 7 days. The isolates were also inoculated on *Aspergillus Flavus* and *Parasiticus* Agar (AFPA) and incubated at 30 °C for 2 days (Pitt and Hocking, 2009).

2.4. Molecular analysis

A total of 57 isolates of *Aspergillus* section *Flavi*, representing different groups according to morphological and physiological characteristics were chosen for more detailed molecular analyses. After growing isolates in liquid Complete Medium (Pontecorvo et al., 1953), the mycelia were collected, frozen in liquid nitrogen and ground to a fine powder. Nucleic acids were extracted using the BioPur Mini Spin Extraction Kit® (Biometrix, Brazil), according to the manufacturers' instructions. DNA amplification of the partial calmodulin (*CaM*) gene region was performed using the cmd5 and cmd6 primer pairs, as described by Hong et al. (2006). Amplicons resulted by PCR were purified using ExoProStar™ 1-Step (GE Healthcare Life Sciences, UK). The amplicons were submitted to direct sequencing in both directions using a BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA) and their products processed in an ABI 3500XL Genetic Analyzer (Applied Biosystems, USA). The *CaM* sequences obtained herein were aligned with the type strains sequences from all *Aspergillus* section *Flavi* species. Phylogeny was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980) with discrete Gamma distribution and invariant sites (G + I), “extensive” (SPR level 5) Subtree-Pruning–Regrafting, and a very weak branch swap filter. *Aspergillus muricatus* was used as outgroup. The analyses were conducted in the software MEGA7 (Kumar et al., 2016) with 1000 bootstrap replicates for assessing node confidences.

2.5. Extrolite analyses

A total of 26 representative isolates were chosen according to their morphological and physiological characteristics. The extrolite analyses were performed according to Frisvad and Thrane (1987) with modifications as detailed by Houbraken et al. (2012). They were grown on both CYA and Yeast Extract Sucrose Agar (YESA) for 7 and 14 days at 37 °C. Five plugs were taken from each medium and the extrolite was extracted with 0.75 mL of ethyl acetate/dichloromethane/methanol (3:2:1) (v/v/v) with 1% (v/v) of formic acid using 50 min

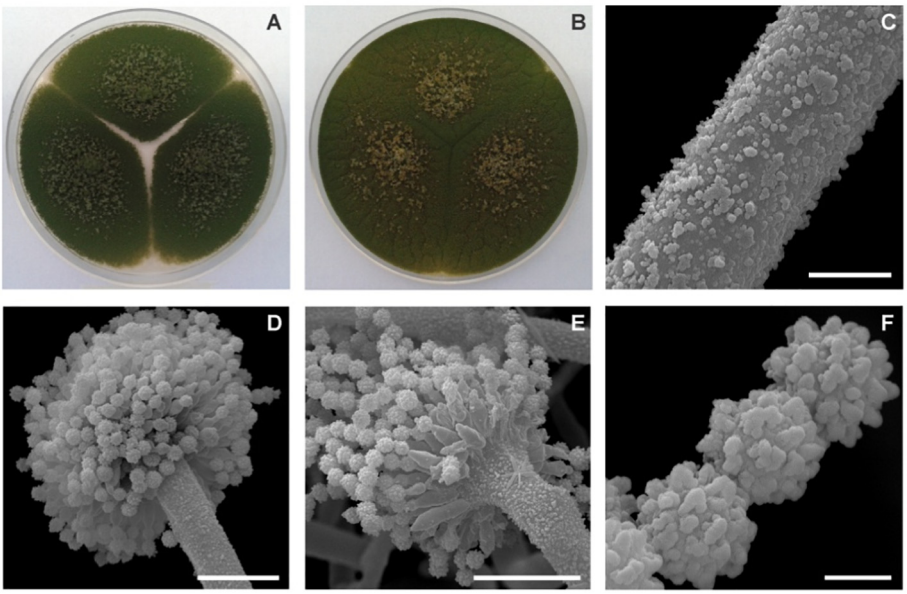

Fig. 1. *Aspergillus novoparasiticus* (ITAL 1) colonies on (A) CYA and (B) MEA, 25 °C, 7 days. (C) Stipe, bar = 5 μm, (D) conidiophore, bar = 20 μm, (E) uniseriate conidiophore, bar = 20 μm and (F) conidia, bar = 2 μm.

ultrasonication. The solvents were evaporated and the dry extract re-dissolved in 0.4 mL methanol. After filtration, the extracts were analyzed using high performance liquid chromatography (HPLC) with diode array detection (Agilent series 1100 system with a Phenomenex Luna C18, USA), according to Nielsen et al. (2011). Authentic standards of the secondary metabolites were used to confirm identity.

2.6. Aflatoxin analysis

2.6.1. Aflatoxin standard

A mixed aflatoxin standard solution (Sigma-Aldrich, USA) was diluted with toluene:acetonitrile (99:1) (v/v) to obtain a calibration curve, in the range 0.005–1.61 ng/μL for aflatoxin B_1, 0.002–0.73 ng/μL for aflatoxin B_2, 0.001–0.45 ng/μL for aflatoxin G_1 and 0.002–0.59 ng/μL for aflatoxin G_2. Five-point calibration curves were plotted with R^2 of 0.999.

2.6.2. Sample extraction and clean-up

Aflatoxin extraction was done using the method of Stroka et al. (2000). Twenty-five grams of a sample was weighed and NaCl (2 g) added. Aflatoxins were extracted with methanol:water solution (100 mL) (8:2) (v/v) for 30 min using a shaker (New Brunswick Scientific, USA). The solution was filtered through Whatman No. 2 filter paper and a Whatman A-Hglass microfiber filter (Whatman, UK). Ten millilitres of the filtrate were diluted with phosphate-buffered saline (60 mL) and applied to an Aflatest WB immunoaffinity column (Vicam, USA) at a flow rate of 2–3 mL per minute. The column was washed with distilled water and aflatoxins eluted with methanol (1250 μL) and diluted with Milli-Q water up to 3 mL.

A Shimadzu LC-10VP HPLC system (Shimadzu, Japan) was used with a fluorescence detector set at 362 nm excitation and 455 nm emission for aflatoxins G_1 and G_2 and 425 nm emission for aflatoxins B_1 and B_2. A Shimadzu CLC G-ODS (4 × 10 mm) guard column and Shimadzu Shimpack (4.6 × 250 mm) column were used. The mobile phase used was water:acetonitrile:methanol (6:2:3) (v/v/v) containing KBr (119 mg/L) and nitric acid (4 M, 350 μl/L). A KobraCell (R-BiopharmRhône, UK) was employed for post-column derivatization. The flow rate of 1 mL per min and current of 100 μA were used. The injection volume was 100 μL.

2.6.3. Methodology optimization

For accuracy, non-contaminated sugarcane juice and dried yeast were spiked with aflatoxins, in triplicate, and the recovery was calculated. For molasses, cream yeast and non-refined sugar, one daily spiked sample was prepared and analyzed with each batch of samples. The spiked level used was 0.98 μg/kg of total aflatoxins. The limits of detection (LOD) and quantification (LOQ) were calculated according to Eurachem Guidelines (2014).

3. Results

3.1. Occurrence of Aspergillus section Flavi in sugarcane, soil and by-products and capacity for aflatoxin production

The number of samples, occurrence, average, median and range of contamination by *Aspergillus* section *Flavi* are shown in Table 1. *Aspergillus* section *Flavi* isolates were dominant. They were found in 70.4% of the sugarcane juice samples, with contamination levels reaching 10^5 CFU/mL, suggesting that they were common in this crop, especially in the São Paulo region of Brazil.

Aspergillus section *Flavi* were also isolated from milled sugarcane, stalk and soil. However, only one isolate was found in the dried yeast. In molasses, unrefined sugar and cream yeast, these species were not isolated at all.

From the total number of samples analyzed, 226 strains of *Aspergillus* section *Flavi* were isolated and the majority, 83.2% (n = 188), came from sugarcane juice, followed by stalk (n = 25), soil (n = 8), milled sugarcane (n = 4) and dried yeast (n = 1). Two main groups of *Aspergillus* section *Flavi* found, were the uniseriate group (n = 191), morphologically very similar to *Aspergillus parasiticus*, and the biseriate group (n = 35). From the biseriate group, only one isolate from dried yeast was identified as *A. flavus*.

All the isolates were tested for aflatoxin production and this showed that 87% (n = 196) were positive. Among these, 99% were B and G aflatoxin producers and 1% was B aflatoxin producers only.

3.2. Aspergillus section Flavi polyphasic identification

Fifty-seven of the *Aspergillus* section *Flavi* isolates, representing the two main groups, were selected and submitted to molecular analyses,

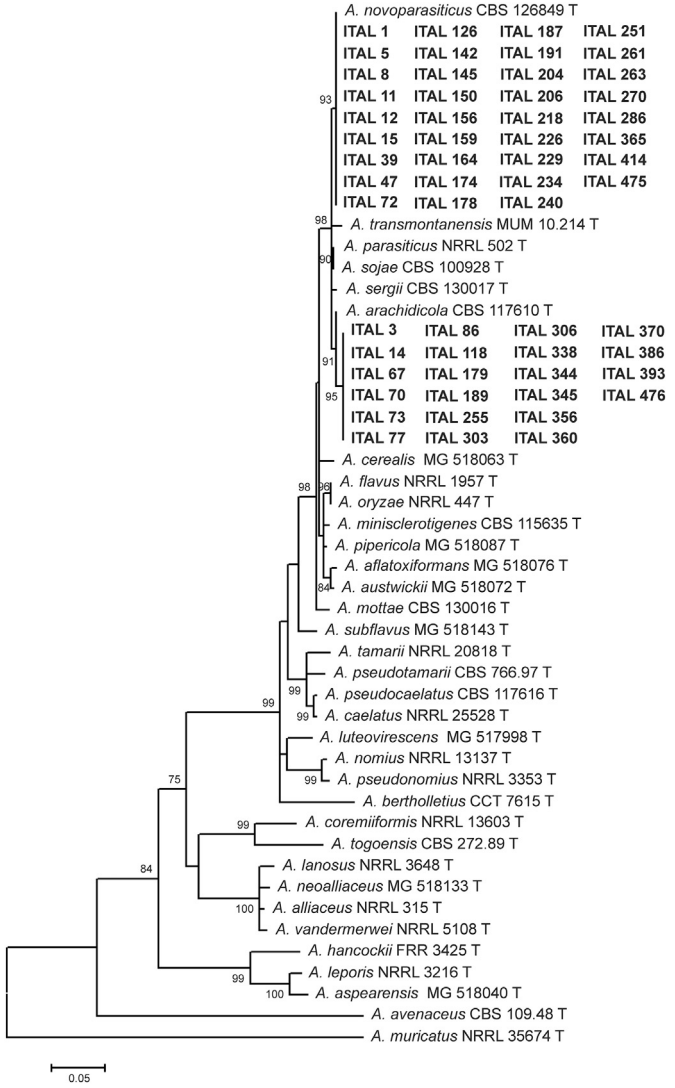

Fig. 2. Maximum likelihood (ML) tree from the partial calmodulin gene sequences aligned with corresponding sequences of type strains from *Aspergillus* section *Flavi*. The bootstrap values (≥70%) are indicated above each node. *Aspergillus muricatus* is the outgroup.

and the following species were identified: *A. novoparasiticus* ($n = 35$) and *A. arachidicola* ($n = 22$). The species most commonly found in sugarcane was *Aspergillus novoparasiticus*. This species was characterized by a green and flat colony (60 mm), uniseriate, small with spherical vesicle (20–25 µm), rough and spherical conidia (5–6 µm) and a rough stipe (20–25 µm) when grown on CYA at 25 °C for 7 days (Fig. 1A). After 7 days incubation, they showed good growth at 37 °C (60 mm) and poor growth at 42 °C (15 mm). They had a yellow/orange reverse and sometimes a weak yellow in AFPA at 30 °C after 48 h. All isolates were able to produce B and G aflatoxins.

A CaM based phylogenetic tree of the *Aspergillus* section *Flavi* was constructed with the sequences obtained in the present study and those from type or neotype strains of all recognized species in *Aspergillus* section *Flavi*, according to Frisvad's update list (Frisvad et al., 2019) as shown in Fig. 2.

Seventeen *Aspergillus novoparasiticus* isolates were analyzed for extrolites and the following compounds were produced: aflatoxins type B and G, ditryptophenalins, kojic acid, miyakamides, parasiticolides, aspirochlorin, aspergilic acid, crysogine and tetracyclic compounds as shown in Table 2. Tetracyclic compounds were only produced by *Aspergillus novoparasiticus* in the *Flavi* group, according to Frisvad et al. (2019). In our study 5 out of 17 strains were not able to produce these compounds.

Although *A. novoparasiticus* was the main species isolated from sugarcane and soil, *A. arachidicola* was also found and was the second most common species from the section *Flavi* group in those isolated from the sugarcane chain. These represented 22 isolates, based on molecular analyses. Nine representative isolates were able to produce aflatoxins type B and G, aspergilic acid, chrysogine, kojic acid, miyakamides and parasiticolide. Only 4 strains (ITAL 344, ITAL 360, ITAL 383 and ITAL 393) were able to produce tenuazonic acid and one strain, versicolorins (ITAL 476). Table 2 shows the extrolites of *A. arachidiola* strains.

3.3. Aflatoxins in sugarcane and by-products

The B_1, B_2, G_1 and G_2 aflatoxins recovery values were 81.6; 79.6; 90.9; 89.7% for sugarcane juice and 84.2; 74.7; 97.9; 65.4% for dried yeast respectively. For molasses, cream yeast and non-refined sugar, the recovery of total aflatoxins varied from 68 to 87%. The results showed the method was satisfactory according to COMMISSION REGULATION CE 401/2006 that established the recovery values from 50 to 120% for aflatoxins level lower than 1 µg/kg. The incidences of aflatoxins in sugarcane and by-products are shown in Table 3.

Most samples, with the exception of sugar, showed some aflatoxin contamination. In the sugarcane juice, one sample had a level of 4.09 µg/kg; however, the median was lower than the limit of detection. In molasses, the average and median values were similar and the contamination ranged from 0.92 to 2.24 µg/kg. Aflatoxins were also detected in 86.4% of cream yeast samples ranging from 0.50 to 0.65 µg/kg. The highest contamination was in dried yeast with an average of 2.55 µg/kg and maximum value of 10.19 µg/kg.

4. Discussion

The presence of *Aspergillus* section *Flavi* in sugarcane stalk and soil samples indicates that the contamination is initiated in the field. These species were not found in molasses and non-refined sugar. Considering the heat treatment which sugarcane juice is processed at to obtain molasses and non-refined sugar, the absence of fungal contaminants in these stages is understandable. Temperature values > 80 °C are achieved at the beginning of the evaporation process and conidia are thermally inactivated. Conidia of different *Aspergillus flavus* and *A. parasiticus* strains were tested and could not survive at temperatures > 65 °C (Doyle and Marth, 1975). Similar results were found with the cream yeast samples, because of the high amount of alcohol in this product. They were directly collected from the fermentation tank, which contains up to approx. 10% alcohol.

The majority of *Aspergillus* section *Flavi* isolates were *A. novoparasiticus* (84.5%). Previous studies have reported *A. parasiticus* (Kumeda et al., 2003; Takahashi et al., 2004; Garber and Cotty, 2014) as being the main species isolated in sugarcane and sugarcane soil ecosystems. However, in our study, *A. parasiticus* was not found, instead *A. novoparasiticus* was the main species isolated in most samples throughout the sugarcane processing chain. This is the first report of *A. novoparasiticus* in sugarcane and sugarcane soil production system. This species was originally isolated from a clinical environment and described by Gonçalves et al. (2012). Subsequently, two *A. novoparasiticus* strains were isolated from cassava samples in Africa by Adjovi et al. (2014), four from corn in Brazil by Viaro et al. (2017) and one isolate from rice by Katsurayama et al. (2018).

A. novoparasiticus is very similar morphologically to *A. parasiticus* and requires DNA sequence analyses for accurate identification. The previous report of *A. parasiticus* isolation from sugarcane in Japan (Kumeda et al., 2003) and Texas (Garber and Cotty, 2014) may actually have been *A. novoparasiticus*. Garber and Cotty (2014) found that both morphology and DNA sequence data of *A. parasiticus* from sugarcane

Table 2
Extrolites produced by *Aspergillus novoparasiticus* and *Aspergillus arachidicola* strains.

Strains	Afla B	Afla G	Kojic acid	Miyakamides	Tetracyclic compounds	Ditryptophenalins	Aspirochlorin	Parasiticolide	Aspergilic acid	Ustilaginoidin	Chrysogine	Tenuazonic acid	Versicolorins
A. novoparasiticus (ITAL 1)	+	+	+	+	+	+		+			+		
A. novoparasiticus (ITAL 5)	+	+	+	+	+	+		+		+			
A. novoparasiticus (ITAL 8)	+	+	+	+	+	+		+	+		+		
A. novoparasiticus (ITAL 11)	+	+	+	+	+	+		+					
A. novoparasiticus (ITAL 12)	+	+											
A. novoparasiticus (ITAL 15)	+	+	+	+	+				+				
A. novoparasiticus (ITAL 39)	+	+	+	+	+	+		+	+		+		
A. novoparasiticus (ITAL 47)	+	+	+	+	+		+	+	+				
A. novoparasiticus (ITAL 126)	+	+	+	+	+		+	+	+		+		
A. novoparasiticus (ITAL 142)	+	+	+	+	+	+		+					
A. novoparasiticus (ITAL 174)	+	+	+	+	+	+		+					
A. novoparasiticus (ITAL 178)	+	+							+				
A. novoparasiticus (ITAL 187)	+	+											
A. novoparasiticus (ITAL 191)	+	+							+				
A. novoparasiticus (ITAL 240)	+	+	+	+		+		+	+				
A. novoparasiticus (ITAL 365)	+	+	+	+	+	+		+	+		+		
A. arachidicola (ITAL 303)	+	+	+					+	+		+		
A. arachidicola (ITAL 306)	+	+	+	+				+			+		
A. arachidicola (ITAL 344)	+	+	+	+				+	+		+	+	
A. arachidicola (ITAL 345)	+	+	+	+		+		+	+		+		
A. arachidicola (ITAL 356)	+	+							+				
A. arachidicola (ITAL 360)	+	+	+	+				+	+		+	+	
A. arachidicola (ITAL 386)	+	+		+				+	+		+	+	
A. arachidicola (ITAL 393)	+	+	+					+	+			+	
A. novoparasiticus (ITAL 475)	+	+	+	+				+	+	+			
A. arachidicola (ITAL 476)	+	+	+	+				+	+		+		+

Table 3
Aflatoxins (μg/kg) in sugarcane and by-products.

Aflatoxins (μg/kg)							
Sample (*n*)	FO (%)[a]		B_1	B_2	G_1	G_2	Total
Sugarcane juice (73)	68.5	Average	0.26	< LD[b]	0.08	< LD	0.43
		Median	< LD	< LD	< LD	< LD	< LD
		Range	ND[c] – 2.25	ND – 1.40	ND – 0.85	ND – 1.26	ND – 4.09
Molasses (24)	100	Average	1.61	< LD	ND	ND	1.72
		Median	1.63	< LD	ND	ND	1.74
		Range	0.86–1.88	0.09–0.14	ND	ND	0.92–2.24
Unrefined sugar (24)	0	Average	< LD	< LD	< LD	ND	< LD
		Median	< LD	< LD	< LD	ND	< LD
		Range	ND – < LD	ND – < LD	ND – < LD	ND	< LD
Cream yeast (23)	86.4	Average	0.47	< LD	ND	ND	0.56
		Median	0.54	< LD	ND	ND	0.55
		Range	0.48–0.62	ND – < LD	ND	ND	0.50–0.65
Dried yeast (54)	73.0	Average	2.00	0.36	0.25	0.18	2.55
		Median	1.80	< LD	< LD	< LD	2.01
		Range	ND – 7.50	ND – 4.44	ND – 2.56	ND – 3.54	ND – 10.19

[a] FO = Frequency of occurrence (number of samples with aflatoxin presence/total number of evaluated samples).
[b] LD = Limit of detection (aflatoxin B_1 = 0.17 μg/kg; B2 = 0.07 μg/kg; G1 = 0.07 μg/kg; G2 = 0.06 μg/kg; total aflatoxins = 0.37 μg/kg).
[c] ND = Not detected.

fields in Texas were more similar to the strains of *A. parasiticus* from Japanese sugarcane fields than those not associated with sugarcane production systems. *A. parasiticus* dominated the *Aspergillus* section *Flavi* community resident on Texas sugarcane and, in so doing, influenced the aflatoxin producing potential of that community and the etiology of aflatoxin contamination of both sugarcane and rotation crops (Garber and Cotty, 2014). However, these studies may not have been aware of the differences between *A. parasiticus* as *A. novoparasiticus*, especially based on molecular evidence (Kumeda et al., 2003; Garber and Cotty, 2014).

A. arachidicola was also isolated from the sugarcane samples in our study. This species was first reported in 2008, isolated from peanuts (Pildain et al., 2008). According to these authors, *A. arachidicola* can produce B and G aflatoxins, kojic acid, chrysogine, parasiticolide and some strains as well as aspergillic acid. Another two compounds, ditryptophenaline and miyakamides were reported later (Varga et al., 2011 and Frisvad et al., 2019). Our results coincided with Pildain et al. (2008) and apart from these, two other compounds were described, tenuazonic acid (four strains) and versicolorins (one strain). Tenuazonic acid has never been reported before as an *A. arachidicola* extrolite. According to Frisvad et al. (2019) only *A. bertholletius*, *A. caelatus*, *A. luteovirescens*, *A. nomius*, *A. pseudocaelatus*, *A. pseudonomius*, *A. pseudotamarii* and *A. tamari* were able to produce it. The diversity of the results can be explained by the differences in the strains and environment where such species can be found.

In Brazil, sugarcane production is economically important and to improve soil productivity, crop rotation between sugarcane and other crops, such as peanuts, is recommended. However, peanuts are very susceptible to infection by *Aspergillus* section *Flavi* species, especially under drought episodes resulting in colonization and aflatoxin contamination (Horn, 2003; Martins et al., 2017) and some studies suggest that composition of *Aspergillus* communities in field soils are influenced by both current and preceding crops (Garber and Cotty, 2014).

The extent to which *Aspergillus* section *Flavi* communities shift during crop rotation is dependent on adaptations of the constituent fungi to the soil environment, including soil sorption characteristics and water potential, soil pH, inoculum potential and survival on crop debris (Jaime-Garcia and Cotty, 2004). However, less information is available on the processes under which specific crops, might favour one species, lineage, or genotype within *Aspergillus* section *Flavi*. Shifts in community composition following rotation of sugarcane with other crops, such as peanuts, may be attributable to plant-created microenvironments that favour *A. parasiticus* or *A. novoparasiticus* and competitive differences among lineages within the section *Flavi* (Garber and Cotty, 2014).

Aflatoxin contamination of sugar cane by-products including creamy and dried yeast may result from contamination by strains of the *Aspergillus* section *Flavi* in the field as well as in the sugar and ethanol processing areas and equipment. Aflatoxins were detected in most samples of sugarcane juice, molasses, cream yeast and dried yeast, although at a low level. The increase on average of aflatoxins in sugarcane molasses may be due to the concentration of its juice by heating to 60–100 °C. This temperature is not enough to cause degradation of the toxins, which are very heat stable (Rustom, 1997; Bullerman and Bianchini, 2007; Raters and Matissek, 2008). The absence of aflatoxins in sugar samples is probably due to degradation after the addition of an alkali agent during the sugar production process. This step is performed to enable a pH correction and for preventing the inversion of sucrose and helping the decantation through flocculation. Aflatoxin degradation was reported by authors using alkaline compounds such as sodium chlorite ($NaClO_2$), sodium hypochlorite (NaClO) and sodium hydrosulfite ($Na_2S_2O_4$) (Tabata et al., 1994; Shi et al., 2017). In summary, this study has clearly shown that the primary source of aflatoxin contamination in dried yeast is from the sugarcane raw material and from the sugarcane juice. It has also been demonstrated that *A. novoparasiticus* is the main species responsible for colonization and aflatoxin contamination during this process. The hypothesis is that during ethanol production aflatoxins can be absorbed into the yeast cellular wall, remaining in the cream yeast and becoming concentrated after the drying process. This is supported by the detection of aflatoxins in all the by-products of ethanol and sugar production, with the exception of the final non-refined sugar itself. Yeast cell walls are known for their ability to bind to aflatoxins and have been previously used as adsorbents to reduce levels of aflatoxins in feed (Fruhauf et al., 2012). This affinity to bind to aflatoxin may explain the higher levels of aflatoxins found in the creamy and dried yeasts during the ethanol and sugar processes. This suggests that more care is needed in terms of quality control in the sugarcane processing chain, especially related to contamination with this new mycotoxigenic species, including better hygiene to minimise the inoculum of *A. novoparasiticus*. In addition, the potential use of the dried yeast as a component of animal feed or as a food supplement needs to be carefully considered to minimise animal exposure to this carcinogenic mycotoxin.

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