Fumonisin-production by *Aspergillus* section *Nigri* isolates from Japanese Foods and Environments

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Fumonisin are well known as mycotoxins produced by various *Fusarium* species. Recently *Aspergillus niger* has been reported to be a fumonisin B₂ (FB₂) producer. *Aspergillus niger* is a member of *Aspergillus* section *Nigri*. Members of this section are common food contaminants and are also distributed widely in the environment. This study aimed to determine 1) optimum culture conditions of *A. niger* for fumonisin production including growth medium, temperature and incubation period and 2) fumonisin production among isolates of *Aspergillus* section *Nigri* and closely related species isolated from Japanese food and environmental samples. Growth on Czapek yeast extract broth +5% NaCl (CYBS) at 28°C for 7 days resulted in the highest levels of FB₂ production as determined by quantitative LC-MS/MS of culture extracts. Sixty-two isolates were collected from various foods in domestic markets as well as from soil and air. The isolates principally separated into two groups; *A. niger* and *A. luchuensis/A. piperis/A. tubingensis*, following molecular phylogenetic analysis. ELISA using the tip culture method was shown to be suitable for screening of the fumonisin-producing strains. Phylogenetic analysis of *Aspergillus* section *Nigri* isolates from food and environmental samples indicated that fumonisin producing strains could be grouped into the *A. niger* clade. Nineteen of 35 (54%) isolates classified as *A. niger* were FB₂ producers. The current study suggests that FB₂-producing *A. niger* are distributed throughout several regions of Japan.

Key words: *Aspergillus niger*, fumonisin B₂, tip culture method, mycotoxin

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Abbreviations: CY20S: Czapek yeast extract-20% sucrose medium, CYA: Czapek yeast extract agar, CYB: Czapek yeast extract broth, CYBS: Czapek yeast extract broth +5% NaCl, CZ: Czapek dox broth, ELISA: enzyme-linked immunosorbent assay, FB₁: fumonisin B₁, FB₂: fumonisin B₂, FB₃: fumonisin B₃, HPLC: high performance liquid chromatography, LC-MS/MS: liquid chromatography-mass spectrometry/mass spectrometry, MEA: malt extract agar, PCR: polymerase chain reaction, PDA: potato dextrose agar, SN: signal to noise, YES: yeast extract sucrose medium

1. Introduction

Mycotoxins, produced by certain fungal contaminants in food and feed commodities, are known to cause food poisoning in humans and domesticated animals¹⁻⁵). One such group of mycotoxins, fumonisins, are produced by *Fusarium* species. Fumonisin B₁ (FB₁) and B₂ (FB₂) were first extracted from cultures of *Fusarium verticillioides* in 1988¹). Fumonisins are now thought to cause leukoencephalomalacia in horses and pulmonary edema in pigs in the United States, South Africa and other areas in the world²). Adverse health implications of exposure to fumonisins have also been suggested in humans. In 1991, long-term animal experiments in rats revealed the onset of liver cancer²) and experiments on mice in 2014 revealed that FB₁ inhibited folic acid transport, which is involved in neural tube closure³). An association has been established between fumonisin contamination in maize cultivated for consumption and increased risk of cancer in humans based on data collected from two geographical regions. These regions include the Transkei region in South Africa, that reported a high prevalence of esophageal cancer⁴), while Haimen county in Jiangsu province, China, saw a high prevalence of esophageal and liver cancer⁵).

Recently, a genome-wide study of *A. niger* revealed the presence of a gene cluster similar to that identified in *Fusarium verticillioides* responsible for fumonisin production⁶) and synthesis of FB₂ has been confirmed in an isolate of *A. niger in vivo*⁷). *Aspergillus niger* has been frequently detected in the environment worldwide as well as in several food products. Owing to its potential for fermentation, the fungus has been used for industrial production of citric acid and oxalic acid. Fumonisins B₂, B₄, and B₆, but not fumonisin B₁ (the more potent toxin), have been reported to be produced during growth on agar-based media of 180 strains of *A. niger*. These included both industrial and laboratory strains, particularly during growth on agar media having low water activity⁸). There have been no reports of fumonisin production other than by *A. niger* and *A. awamori*⁹) (*A. awamori* is no longer used as the kuro-koji mold for shochu-brewing) in *Aspergillus* section *Nigri*, a closely related group of fungi that resembles the characteristics of *A. niger* both morphologically¹⁰) and genetically.

Outside of Japan, contamination of food products with FB₂, which is surmised to result from contamination with *A. niger*, has been reported in grape juice¹¹), wine¹²), and dried grapes¹³). Moreover, *A. niger* strains isolated from dried grapes¹⁴) were reported to produce FB₂ up to maximum 7.841 ppm. Thus, the occurrence of FB₂ contamination in food products by *Aspergillus* section *Nigri* strains, in particular *A. niger*, is becoming more apparent. However, in Japan, there

have been no reports of detection of FB₂ in food products due to contamination with *Aspergillus* section *Nigri* isolates such as *A. niger*. Additionally, FB₂ production by food product-derived strains of these fungi is limited to a single report of an isolate from imported, dried figs¹⁵). Thus, the prevalence of FB₂ contamination of domestically distributed food products in Japan by *Aspergillus* section *Nigri* isolates is unclear, and it is hence essential to perform a detailed survey of fumonisin-producing strains of this *Aspergillus* section.

In the present study, we investigated the optimal growth and screening conditions for fumonisin-producing strains of *Aspergillus* section *Nigri*, and we collected isolates of this section from Japanese commercial food products and environments and determined their potential for fumonisin production.

2. Materials and Methods

2-1. Isolation of Fungal Strains from Foods and Environments

Aspergillus section *Nigri* strains were isolated from 15 foods and 11 environmental samples. Details of the food samples were as follows: polished rice, onion, wheat, soybeans, passion fruit, pickled perilla, bread, pepper imported from southeast Asian and corn grits imported from the United States. All food samples were purchased from Japanese markets. Details of the environmental samples were as follows: soil, wood, horse stall straw, and air collected in several areas of Japan (summarized in **Table 1**). Samples were placed on Dichloran Glycerol (DG-18; Kanto Chemical Co. Ltd., Tokyo, Japan) agar plates supplemented with chloramphenicol (Wako, Osaka, Japan) at 100 mg/ml. For collection of air-borne samples, an air sampler (air ideal 3P; Sysmex Biomelius, Tokyo, Japan) was used. Agar plates were incubated at 25°C for 7-10 days in the dark. Following incubation, fungal colonies were visually observed and those of *Aspergillus* section *Nigri* were collected as black aspergilli and then plated on potato dextrose agar (PDA; Eiken Chemical Co., Tokyo, Japan).

2-2. Identification of *Aspergillus* section *Nigri* Strains

Isolates of *Aspergillus* section *Nigri* were identified using morphological and molecular techniques. For morphological identification, isolates were transferred to Czapek agar medium (Cz: Czapek Dox Broth, Becton Dickinson and Company, France), 12 g agar (Shoei Co., Tokyo, Japan) per liter, malt extract agar medium (MEA: Kanto Chemical Co., Ltd.), and Czapek-yeast extract agar medium (CYA: per liter: Czapek Dox Broth, 12 g; Bacto Yeast Extract (Difco Becton

Table 1. Number of isolated strains, detected fumonisin B₂^{*1}, and range/average yields of fumonisin B₂

Origin	Clade*	Number of isolated strain	Number of fumonisin positive from ELISA method	Number of fumonisin positive samples (LC/MS/MS)	Range ^{*4} of fumonisin B ₂ concentrations LC-MS/MS (ppb)	Average ^{*4} of fumonisin B ₂ concentrations LC-MS/MS (ppb)
Food	Pepper	<i>A. niger</i> ^{*2}	17	10	100 - 3500	1100
		<i>A. luchuensis/A. piperis/A. tubingensis</i> ^{*3}	4	0	—	—
	Corn grits	<i>A. niger</i>	3	3	1550 - 2350	1950
		<i>A. luchuensis/A. piperis/A. tubingensis</i>	1	0	—	—
	Domestic Rice	<i>A. niger</i>	8	8	1500	1500
		<i>A. luchuensis/A. piperis/A. tubingensis</i>	7	0	—	—
	Wheat	<i>A. luchuensis/A. piperis/A. tubingensis</i>	1	0	—	—
	Soy beans	<i>A. niger</i>	1	1	ND ^{*5}	ND
		<i>A. luchuensis/A. piperis/A. tubingensis</i>	1	0	—	—
	Onion	<i>A. niger</i>	4	3	ND	ND
	Bread	<i>A. niger</i>	1	0	—	—
	Passion fruit	<i>A. niger</i>	1	1	ND	ND
Environment	Pickle of red perilla	<i>A. luchuensis/A. piperis/A. tubingensis</i>	1	0	—	—
		Subtotal	50	26 (52%)	10 (20%)	
	Soil	<i>A. niger</i>	1	0	—	—
	Wood	<i>A. niger</i>	2	1	280	280
	Straw	<i>A. luchuensis/A. piperis/A. tubingensis</i>	1	0	—	—
	Air	<i>A. niger</i>	5	3	100 - 310	205
		<i>A. luchuensis/A. piperis/A. tubingensis</i>	3	0	—	—
		Subtotal	12	4 (33%)	3 (25%)	
		Total	62	30 (48%)	13 (21%)	

^{*1}Fumonisin B₁ and B₃ were not detected.^{*2}*Aspergillus niger*^{*3}*Aspergillus luchuensis*, *Aspergillus piperis*, or *Aspergillus tubingensis*^{*4}Only fumonisin-producing strains were involved.^{*5}<2.5 ppb

Table 2. Reference sequences of beta tubulin from database

GenBank accession no.	Taxon name	Strain number
AB574097.1	<i>Aspergillus awamori</i> (re-identified “ <i>Aspergillus niger</i> ”)	RIB 2603
AB574111.1	<i>Aspergillus awamori</i> (re-identified “ <i>Aspergillus niger</i> ”)	NBRC 8877
GU296687.1	<i>Aspergillus niger</i>	CBS 554.65
AB574102.1	<i>Aspergillus niger</i>	RIB 2611
AY820006.1	<i>Aspergillus brasiliensis</i>	CBS 101740
FJ629280.1	<i>Aspergillus foetidus</i>	CBS 564.65 ^T
FJ629303.1	<i>Aspergillus piperis</i>	CBS 112811 ^T
GU296699.1	<i>Aspergillus costaricensis</i>	CBS 115574 ^T
FJ629305.1	<i>Aspergillus tubingensis</i>	CBS 134.48
FJ629276.1	<i>Aspergillus carbonarius</i>	CBS 111.26 ^T
AY819992.1	<i>Aspergillus flavus</i>	CBS 100927 ^T
EF203128.1	<i>Aspergillus caelatus</i>	CBS 117616

Dickinson and Company), 2 g). Plates were incubated at 25°C for 7 days and then the isolates were morphologically identified based on colony textures and microscopic observation according to Susca et al.¹¹⁾ For molecular identification, isolates were inoculated into 1 ml potato dextrose broth (PDB; Difco Becton Dickinson and Company) in 2.0 ml microtubes and incubated overnight at 25°C. After incubation, the fungal mycelia were collected by centrifugation at 15,000 rpm for 10 min at 4°C and the genomic DNA were extracted using the DNeasy Plant Mini Kit (QIAGEN, The Netherlands) according to the manufacturer’s instructions. The β -tubulin gene (β -*tub*) was amplified from the extracted genome for purposes of β -*tub* phylogenetic analysis¹⁶⁾. PCR reactions were performed using TaKaRa *Ex Taq* (Takara Bio Co., Ltd., Otsu, Japan) with Bt2a (GGTAAC-CAAATCGGTGCTGCTTTC) and Bt2b (ACCCTCAGT-GTAGTGACCCTTGGC)¹⁶⁾ as primers. The thermocycler parameters consisted of a denaturing step at 94°C and 3 min, followed by amplification for 35 cycles (94°C for 30 sec, 52°C for 40 sec, and 72°C for 1.5 min). The final elongation reaction was performed at 72°C for 5 min. PCR products were purified using ExoSAP IT (GE Healthcare Japan, Tokyo, Japan) according to the manufacturer’s instructions. DNA sequencing reactions were performed using BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA) with Bt2a or Bt2b as primers. The PCR products were purified according to a protocol published by Thermo Fisher Scientific and sequenced using a 3730 XL genetic analyzer

(Thermo Fisher Scientific, USA). The sequences were edited using ATGC ver. 6.0 (Genetycs, Tokyo, Japan). All β -tubulin sequences were phylogenetically analyzed with the β -tubulin sequences of *Aspergillus* reference strains downloaded from GenBank. The references are described in **Table 2**. In this study, two strains, RIB 2603 and NBRC 8877, which are provided as *Aspergillus awamori*, were described as *Aspergillus awamori* re-identified “*Aspergillus niger*”, because both strains were re-identified as *Aspergillus niger*¹⁷⁾. Sequences were aligned and analyzed using MEGA6¹⁸⁾. Phylogenetic analysis was performed by the neighbor joining method with the maximum composite likelihood model. The stability of clades was assessed with 500 bootstrap replications.

2-3. Fumonisin Production by *Aspergillus* Section *Nigri* Strains

In this study a simple and rapid “Tip Culture” method was adopted for fungal growth and preparation of culture solutions for toxin analysis¹⁹⁾. The optimum substrate, temperature and time of incubation that provided the greatest amounts of fumonisins during growth of fungal strains was ascertained. Specifically, five types of liquid medium were prepared that had been found to be highly productive for fumonisin production in *A. niger*⁷⁾; CYB (CYA broth), CY20S broth (CYB + 20% sucrose), YES broth (Bacto Yeast Extract+sucrose+MgSO₄ · 7H₂O), PDB, and CYBS broth (CYB + 5% NaCl). *Aspergillus niger* MAFF425037, which was previously shown to produce FB₂, was incubated

at 25°C statically for 7 days. After incubation, the spores were collected in PBS containing 0.1% tween 80 for use as an inoculum. Three µl of fungal spores (about 10^3 spores/µl) were used to inoculate each 300 µl of broth present in the tip culture set up used. Cultures were incubated at 25°C for 30 days in the dark. Following incubation, 20-fold dilution of each broth was prepared for analysis of tip FB₁, FB₂ and FB₃ by LC-MS/MS. Analyses were performed using a LC-20A HPLC (Shimadzu Corporation, Kyoto Prefecture) and 3200 Q TRAP mass spectrometer (AB Sciex, Redwood City, CA). Samples were loaded onto an Inertsil ODS-4 column (3.0×50 mm, 2 m; GL Sciences) at 40°C. The mobile phase was a binary gradient of solvent A (0.1% formic acid in water) and solvent B (acetonitrile) programmed as follows: at 0 min, 25% B; at 5 min 50% B; at 8 min, 50% B. The flow rate was set at 0.2 mL/min. Ion monitoring were as indicated below; precursor m/z > product m/z, fumonisin B1 722 [M+H]⁺>352, fumonisin B2 706 [M+H]⁺>336, and fumonisin B3 706 [M+H]⁺>336. The concentration of 0.3 ppb, which is equivalent to an SN ratio of 3 at the time of measurement of fumonisins reference standard, was set as the lower limit of detection, and the concentration of 1 ppb, which is equivalent to an SN ratio of 10, was set as the lower limit of quantification. Cultures were incubated at 25°C, 28°C, and 32°C for 3, 5, and 7 days on the various media using the tip culture technique. Diluted samples were analyzed for FB₁, FB₂ and FB₃ by LC-MS/MS. These experiments were repeated twice to calculate the mean of the results obtained.

2-4. Screening of Fumonisin-Producing Strains

Tip culture broths were screened for production of total fumonisin using an Enzyme-Linked Immuno Sorbent Assay (ELISA)-based Veratox Fumonisin Quantitative Test Kit (NEOGEN, U.S.A.). Microplate Manager 6 (Bio-Rad Laboratories, Tokyo, Japan) was used to measure the absorbance at a 630 nm wavelength. Samples giving a value of 2.5 ppb or higher total fumonisins were judged as positive for fumonisin production by this screening method.

2-5. Quantitation of Fumonisin Production

Strains that tested positive for fumonisin production by ELISA were quantitatively analyzed for fumonisin levels by LC-MS/MS. A 10- to 500-fold dilution of the samples was used for LC-MS/MS. Although the measurement conditions were the same as those described above, a threshold value for a sample considered positive for fumonisin production by LC-MS/MS was also set at 2.5 ppb or higher as this value is the same as the lower limit of detection in the ELISA method.

3. Results

3-1. Optimal Culture Conditions for Fumonisin B₂ Production in *Aspergillus* section *Nigri*

Fumonisin B₂ production by *Aspergillus* section *Nigri* strains was compared using 5 types of liquid culture media at 25°C for 7 days. The amounts detected by LC-MS/MS were low in the CYB, CY20S, YES, and PDB at 54 ppb, 3 ppb, 8 ppb, and below the limit of detection, respectively; however, in CYBS, 600 ppb FB₂ was detected. Thus, CYBS was found to be optimal among the 5 liquid culture media tested. Furthermore, the temperature and duration of culture for fumonisin production were investigated using CYBS culture medium. Culture temperatures of 25°C, 28°C, and 32°C were used with incubation periods of 3, 5 and 7 days. The maximum production of FB₂ was detected by LC-MS/MS when cultures were grown at 28°C for 7 days, with an average of 84.5 ppb FB₂.

The tip culture method¹⁹⁾ was used previously to investigate aflatoxin production in *Aspergillus* Section *Flavi*; however, when used with a liquid CYBS culture medium, it was found to be suitable for detection of FB₂ production in *Aspergillus* section *Nigri*, and as a rapid detection method for fumonisin-producing strains.

3-2. Efficacy of Screening Methods via ELISA

Screening for fumonisin production was performed for 62 *Aspergillus* section *Nigri* strains, of which 30 were found to produce FB₂ by ELISA. Quantitative LC-MS/MS of ELISA positive cultures, found that FB₂ was detected in the range of 100 to 3500 ppb in culture broths of 14 of these strains, while the remaining were false-positives. Fumonisin B₁ and FB₃ were not detected in any of the strains. In addition, LC-MS/MS was performed on broths for detection of FB₂ from 22 strains that tested negative for FB₂ production by ELISA. There were no samples that exhibited amounts above the detection level of 2.5 ppb, indicating that the ELISA method does not produce false-negatives. The false-positive results may have been the result of cross-reactivity with intermediate metabolites with a similar structure to FB₂ in the antigen-antibody reaction, enzyme reaction, or color-inducing steps in ELISA. However, since no false-negatives occurred with this method, it seems effective as a means of initially screening cultures for the ability to produce fumonisins.

3-3. Phylogenetic Relationships of Fumonisin-Producing Strains

Sixty-two strains were isolated from the environment or from food products and the nucleotide sequences of *β-tub* of these strains were determined in this study (GenBank ac-

Fig. 1. Phylogenetic tree of strains of the genus *Aspergillus* derived from environment/food in Japan based on β -tubulin nucleotide sequence. The text of each node shows the species, the origin of each strain noted in parentheses, and the strain number, respectively. Sequences were aligned and analyzed using MEGA6. Phylogenetic analysis was performed by neighbor joining method with the maximum composite likelihood model. The stability of clades was assessed with 500 bootstrap replications. Nodal numbers indicate bootstrap probabilities. The branch lengths are proportional to the numbers of nucleotide substitutions. The scale bar shows the number of substitutions per site. The sequences of *A. flavus* and *A. caelatus* were used as outgroups.

cession Nos. LC387849-LC387876, LC389050-LC389061). Each stain was classified based on both morphological observations and molecular phylogenetic analysis. A phylogenetic tree based on nucleotide sequences of β -*tub* (331bp), inferred using the neighbor joining method, is shown in **Fig. 1**. The bootstrap values were highly maintained, at 100% for the *A.*

niger clade, and 96% for the clade containing *A. luchuensis*, *A. piperis*, *A. costaricensis* and *A. tubingensis* (herein referred to as the *A. luchuensis/A. piperis/A. tubingensis* clade). The β -*tub* locus did provide good support (86% bootstrap) for delineating the *A. tubingensis* clade, but further inference of relationships among the species within the *A. luchuensis*

/A. piperis/A. tubingensis clade was not well supported. This result was consistent with the previously reported phylogenetic analysis of Hong et al.¹⁷⁾ based on nucleotide sequences of the rDNA-ITS region, *β-tub*, and calmodulin gene.

3-4. Fumonisin-Producing Status of *Aspergillus* section *Nigri*

Table 1 summarizes fumonisin production of fungi based on origin and the surmised clade of each strain. Of the 62 strains, 43 (69%) were *A. niger*, with the rest classified into the *A. luchuensis /A. piperis/A. tubingensis* clade. Among the 62 strains assessed, 13 (21%) were positive for FB₂ production in accordance with LC-MS/MS results, with all of them being *A. niger*. Fumonisin producing-strains comprised 30% of the 43 strains that were classified into the *A. niger* clade. In contrast, fumonisin production was negative in all 19 strains classified into the *A. luchuensis /A. piperis/A. tubingensis* clade.

When examining the strains by origin, 17 of the 21 *Aspergillus* section *Nigri* strains isolated from imported pepper were identified as *A. niger*, of which seven were found to produce fumonisin (41% of the isolated *A. niger* strains). Of the four *Aspergillus* section *Nigri* strains isolated from imported corn grit, three were identified as *A. niger*, of which two were found to produce fumonisin (67% of the isolated *A. niger* strains). Of the 15 *Aspergillus* section *Nigri* strains isolated from domestic rice, eight were identified as *A. niger*, of which one produced fumonisin (13% of the isolated *A. niger* strains). Of the six *Aspergillus* section *Nigri* strains isolated from domestically produced dried soy bean, onion, and passion-fruit food products, five were identified as *A. niger*; however, none of these produced fumonisin. Of the eight *Aspergillus* section *Nigri* strains isolated from the air, five were identified as *A. niger*, of which two were found to produce fumonisin (40% of the isolated *A. niger* strains). Of the two *Aspergillus* section *Nigri* strains isolated from woods, both were identified as *A. niger* and one was found to produce fumonisin (50% of the isolated *A. niger* strains). In this study, the FB₂-producing strains were found to belong only to the *A. niger* clade.

Based on their origin, FB₂ production was often higher for strains derived from foods, such as pepper, corn grit, and rice while FB₂ production of strains isolated from the environment tended to be lower under the conditions used in this study.

4. Discussion

Fumonisin B₂ production by isolates of *Aspergillus* section *Nigri* had only been confirmed in *A. niger* strains originating from imported dried figs in previous studies of food products distributed in Japan¹⁵⁾. However, the present study demonstrates that a considerable proportion, 33% of the *A. niger* strains derived from various food products, produce FB₂ regardless of whether they are of Japanese origin or imported from other countries. While the production of fumonisins is not as high as that detected in fungi of the genus *Fusarium*, it seems possible that many food products can potentially be contaminated with FB₂ from *A. niger*. Future studies are required to investigate the distribution of FB₂-producing *Aspergillus* section *Nigri* strains in nature, particularly *A. niger*, and the risk posed by them in food products with a wide distribution including in Japan.

In addition, the tip culture method used in the present study has been reported as a suitable method for verifying aflatoxin production in *A. flavus*¹⁹⁾. To our knowledge, this is the first time its efficacy has been verified in experiments to confirm fumonisin production in *A. niger*. Agar culture media are often used for detecting fumonisin production in *A. niger* but the process of extraction of the culture media and preparation of samples for analysis can be quite laborious. In contrast, using the tip culture system, incubation is performed in a small incubator and collection of the culture fluid requires only a single centrifugation step. Because sample broths can be rapidly obtained from multiple strain cultures, it is a promising method for high throughput experiments requiring analysis of numerous samples.

Mycotoxin productivity is species-specific to some extent, with particular mycotoxins associated with certain genera, and the presence of mycotoxins in commodities can be considered an indicator of infection and growth of the producing fungi²⁰⁾. Furthermore, phylogenetic relationships can, in some cases, be used to determine mycotoxin production capability in fungi that have not been evaluated for their mycotoxigenicity²¹⁾. Such predictive inferences are potentially useful for monitoring and management of mycotoxin exposure. According to previous reports, the *A. niger* and *A. luchuensis /A. piperis/A. tubingensis* groups are each very closely related with similar morphologies^{17,22)}, and in the present study, fumonisin production was only observed in the *A. niger* clade. The difference in FB₂ production between the *A. niger* and *A. luchuensis /A. piperis/A. tubingensis* clades may be due to genetic defects that result in loss of expression or the genes related to fumonisin biosynthesis²³⁾; however, these details remain unclear.

Additionally, the *A. niger* clade contained a mixture of

fumonisin-producing groups and non-producing groups. However, in the Okinawa region, several fungal strains used in brewing since antiquity form a group that is clearly distinct even among the other *Aspergillus* section *Nigri* strains. Moreover, these strains are non-fumonisin producers²⁴). It may be that domestication of fumonisin producers led to artificial selection of strains for brewing with a low potential for fumonisin production. In contrast, *A. niger* that has been selected and used industrially for production of citric acid exhibits the same level of fumonisin production as naturally derived strains⁹). Therefore, the elucidation of the detailed mechanism of fumonisin production by fungi of the *Aspergillus* section *Nigri* clade is clearly needed.

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Conflict of interest

The authors have no conflict of interest.

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