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SHORT COMMUNICATION

Fumonisin B_2 by *Aspergillus niger* in the grape–wine chain: an additional potential mycotoxicological risk

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Abstract Fumonisins are mycotoxins with cancer-promoting activity and are associated with a number of animal and human diseases. The potential risk of contamination by fumonisin B_2 (FB_2), although at low levels, has been demonstrated in must and wine. Black aspergilli in general and *Aspergillus niger* in particular are considered to be the major responsible agents of FB_2 contamination in grape and its by-products. Contamination by FB_2 therefore is yet another safety concern of grape and wine producers, as ochratoxin A, produced mainly by *A. carbonarius*, may prove to be a major mycotoxicological problem in the grape–wine chain.

Keywords Fumonisins · Mycotoxins · *Aspergillus* · Wine

Fumonisins have been extensively studied as mycotoxins produced by *Fusarium* species that have cancer-promoting activity (Gelderblom et al. 1991) as well as being associated with a number of animal and human diseases (Harrison et al. 1990; Marasas 2001). The fumonisin B (FB) molecule is composed of a long hydroxylated hydrocarbon chain with added tricarballylic acid and methyl and amino groups (Gelderblom et al. 1991). FB_1, FB_2, and FB_3 are the major naturally occurring FBs. FB_1 is by far the most prevalent of the fumonisins in the human diet and is categorized as a Group 2B carcinogen by the International Agency for Research on Cancer (IARC 2002). Studies in the former Transkei region of South Africa and in the Linxian and Cixian counties, China, have demonstrated an association between exposure to fumonisins in rural subsistence farming areas and a high incidence of esophageal cancer (Rheeder et al. 1992; Zhang et al. 1997). Fumonisins, which inhibit the uptake of folic acid via the folate receptor (Stevens and Tang 1997), have also been implicated in the high incidence of neural tube defects in rural populations known to consume contaminated maize, such as in the former Transkei region of South Africa and some areas of Northern China (Marasas et al. 2004).

Frisvad et al. (2007) recently reported that a number of industrially important strains of *Aspergillus niger* also produce FB_2 and FB_4 (Fig. 1), additionally pointing out the potential mycotoxicological risk of some foods colonized by this species. Based on these findings, Logrieco et al. (2009) evaluated the possibility of fumonisin contamination in must from grapes naturally contaminated by black *Aspergillus* in Apulia, a region in southeastern Italy. Of the 12 must samples tested by this group, two contained FB_2 at trace levels and 0.4 μg/mL, respectively. When these researchers evaluated 31 representative strains belonging to four *Aspergillus* species commonly found on grape for fumonisin production on agar plates, eight of the ten strains of *A. niger* produced FB_2 (range 0.1–293 μg/g), while none of the strains of *A. uvarum* (7)*, A. tubingensis*, (8), and *A.*

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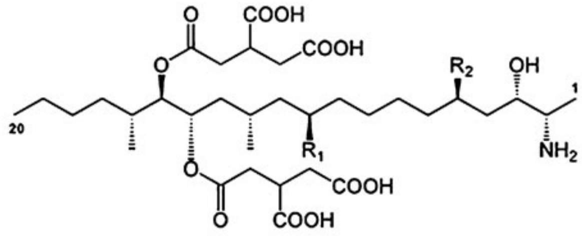

Fig. 1 Chemical structures of fumonisins B_2 (*FB_2*) and B_4 (*FB_4*)

carbonarius (8) produced detectable amount of fumonisins. Mogensen et al. (2010a) recently demonstrated that *A. niger* strains isolated from raisins are capable of producing both FB_2 and FB_4 when cultured on either grapes or raisins. In yet another study, examination of 48 strains of *Aspergillus* section *Nigri* for the presence of an ortholog of FUM8, which serves as a marker for the fumonisin biosynthetic gene cluster, detected only 11 *A. niger* strains with the fum8 gene, nine of which were also FB_2-producing strains (Susca et al. 2010). These findings indicate a certain discontinuous distribution of fumonisin-producing *A. niger* strains and that the absence of fumonisin production is associated—at least in part—with the fumonisin biosynthetic gene cluster. However, these findings also indicate that there is a potential risk of consumer exposure to FB_2 in the grape–wine chain and that *A. niger* may represent the major fumonisin-producing species among black Aspergilli occurring on grapes.

This hypothesized risk of FB_2 being present in wine was confirmed by a liquid chromatography–tandem mass spectrometry analysis of 51 market samples (45 red wines, 5 white wines, one rosé wine) of wine produced in various Italian regions (Logrieco et al. 2010). Nine samples (17%) of red wine were found to be contaminated by FB_2 at levels ranging from 0.4 to 2.4 ng/mL, while FB_4 was not detected in any of the tested samples. This risk was confirmed by a contemporary study by Mogensen et al (2010b) in which FB_2 was detected in 18 (23%) of 77 wine samples from 13 different countries at levels ranging from 1 to 25 ng/mL. Although these findings are based on limited number of tested wine samples, it would appear that red wine is more susceptible to contamination than white wine, similar to ochratoxin A (OTA) contamination.

Regulations concerning FBs contamination are in place in most developed countries. The European Mycotoxin Awareness Network (EMAN 2000) has set a “safe dose”, called the “provisional-maximum-tolerable-daily-intake” [PMTDI; μg/kg body weight (BW) per day], for humans, which was obtained by dividing the “no-observed effect level” (NOEL, mg/kg BW per day) by a safety factor extrapolated from animal experiments.

The joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee for Food Additives has also set a provisional maximum tolerable daily intake of 2 μg/kg BW per day for FB_1, FB_2, and FB_3 alone or combined (FAO/WHO 2002). This “dose” is based on a no-observable-adverse-effect level in the male rat kidney and incorporates a 100-fold safety factor. Within the European Union, the Commission Regulation (European Commission 2007) has established maximum limits for the sum of FB_1 and FB_2 in foodstuff; more specifically, there is a maximum level of 1,000 μg/kg for corn intended for direct human consumption, with the exception of corn-based breakfast cereals and snacks (800 μg/kg) and processed corn-based foods and baby foods for infants and young children (200 μg/kg). However, in many of the high-maize consumption areas of the world, regulations are either lacking or not enforced. For Italian consumers, Brera and Miraglia (2003) calculated a fumonisin intake of 185.6 ng/kg BW/day from the consumption corn-derived products, 62.1 ng/kg BW/day for wheat products, and 283.6 ng/kg BW/day for corn fractions. A risk to fumonisin exposure can be escalated by alcohol consumption. In the USA, the contribution of fumonisins from beer to the overall dose appears to be rather small (20–54 ng/kg BW per day, calculated on the basis of a body weight of 60 kg) (Hlywka and Bullerman 1999). However, the heavy beer drinker, depending upon the amount of fumonisin contaminated corn used, may be exposed to an increased risk (Kuiper-Goodman et al. 1996). Assuming a daily consumption of 200 ml of wine for a 70-kg individual and a contamination level of FB_2 equal to the average of the positive samples of 1 ng/mL (Logrieco et al. 2010), the average exposure to fumonisins through wine consumption would be 0.003 μg/kg BW per day. Therefore, the contribution of wine to mean daily intake of fumonisins can be considered negligible in the case of wine drinkers. In terms of fumonisin exposure, wine consumption can therefore only become a cause of concern when it is combined with a contaminated maize-based staple diet, as maize is generally the major fumonisin-contaminated crop.

Aspergillus niger and *A. carbonarius* have been frequently isolated together from grapes grown in Europe (Sage et al. 2002, Battilani et al. 2006; Belli et al. 2006; Tjamos et al. 2006), Australia (Leong et al. 2004), and South America (Chulze et al. 2006), and they represent the major fungi responsible for FB_2 and OTA production in grape wine, respectively. The potential risk of FB_2 is a realistic one when consideration is given to the fact that *A. niger* aggregates are consistently present on grapes during the growing season, when they are generally present at higher levels than other black aspergilli. These two species show different ecological behavior. *A. carbonarius* and *A niger* aggregates differ in their temperature optima for growth, with optimal growth occurring at 25–30°C for the former and at 30–35°C for the latter. Further studies are needed to better define the environmental conditions and other factors influencing the production of the toxin, determine the fate of fumonisins during the winemaking process, and accurately identify *Aspergillus* species in the Section *Nigri*. It is very possible that the toxic profile of any single species is different, thereby exposing consumers of the contaminated food commodities to different toxicological risks (Logrieco et al. 2009).

In conclusion, this report provides new information on the mycotoxin risk represented by the presence of fumonisins in wine and the importance of being able to control *Aspergillus* species occurring on grape, confirming that these species could pose a potential risk for human and animal health.

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