



Review

Control of ochratoxin A-producing fungi in grape berry by microbial antagonists: A review

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ARTICLE INFO

Article history:

Received 24 September 2015

Received in revised form

11 March 2016

Accepted 26 March 2016

Available online 30 March 2016

Keywords:

Grapes

Fungi

Biological control

Postharvest decay

Ochratoxin A

Detoxification

ABSTRACT

Background: Ochratoxin A (OTA) remains a challenge in the face of continuous efforts to produce quality and wholesome wine and table grape berries to meet food safety standards. However, the use of chemicals to control postharvest diseases is a public concern with increasing consumer awareness of the dangers associated with the consumption of fungicide-treated commodities. Synthetic fungicides are well-known for their hazardous effects on human health and potential contamination to the environment. Moreover, pathogens are noted to have developed resistance to these chemicals because of their continuous use and abuse.

Scope and approach: This review focused on the efficacies, potentials and developmental trends of microbial antagonists in the control and biodegradation of OTA-producing fungi in grapes and wine. It outlined the steps and challenges in the development of bioproducts. It also recounted the successes and developments of biocontrol products to date.

Key findings and conclusions: OTA production in grapes is caused by the genera *Aspergillus* and *Penicillium*, with the species *Aspergillus carbanarius* as the dominant cause across the globe. The prevalence of OTA-producing fungi in grape vineyards are influenced by temperature, water activity (a_w), pH, relative humidity and type of cultivar. Biological control agents (BCAs) have proved successful to control and/or degrade OTA-producing fungi, among which antagonistic yeasts play the leading role. In addition, biocontrol products such as BioSave, Yieldplus, Serenade and Aspire have been developed. Preharvest application is acknowledged to be the best for grapes even a day before harvest because postharvest treatment affects the bloom of the grapes.

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1. Introduction

OTA is among the most important mycotoxin contaminants of foodstuffs and beverages due to its potent immunotoxic, teratogenic, nephrotoxic and genotoxic properties to humans (Pfohl-Leschkowicz, 2007). OTA has been categorized as a possible human carcinogen (group 2B) according to the International Agency of Research on Cancer (IARC, 1993). Notably, *Aspergillus ochraceus* and *Penicillium verrucosum* were considered to be the main cause of OTA in tropical and temperate regions respectively (Mantle, 2002). However, apart from *Penicillium nordicum* and *Penicillium verrucosum*, the genus *Aspergillus* is considered the most important species accountable for the presence of OTA in food. This genus is reported to have more than 100 species, with a complex taxonomy under

continuous revision. It was last revised and published based on broad phylogenetic analysis (Peterson, 2008). OTA is a common contaminant of a wide range of food and food products which include cereals and its derived products (Jørgensen & Jacobsen, 2002), grapes, cocoa beans, spices, nuts, olives, grapes, beans coffee beans and figs (Battilani, Pietri, Bertuzzi, Languasco, Giorni, & Kozakiewicz, 2003). Grapes and its derived products are the second most contaminated with OTA after cereals. Table 1 list of OTA-fungi found in grapes. Regarding meat, OTA has been reported in non-ruminants such as pigs, poultry, rabbits and rats (Fink-Gremmels, 2008). Ruminants on the other hand, tolerate OTA because of the protozoan normal flora of their rumens which degrades OTA into OTA α . Nonetheless traces of OTA have been found in meat from ruminants and dairy milk.

Based on the health risks associated with OTA, the European Union in 2006, passed a legislation recommending the maximum permissible limits of OTA in a number of produce (De Curtis, de Felice, Ianiri, De Cicco, & Castoria, 2012). Numerous researches

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Table 1
OTA-producing fungi found in grapes.

Fungi	References
<i>Aspergillus carbanarius</i>	(Bellí et al., 2007).
<i>Aspergillus ochraceus</i>	(Gil-Serna, González-Salgado, González-Jaén, Vázquez, & Patiño, 2009).
<i>Aspergillus niger</i>	(Esteban et al., 2004).
<i>Penicillium nordicum</i>	(Selma, Martínez-Culebras, & Aznar, 2008).
<i>Penicillium verrucosum</i>	(Battilani & Pietri, 2002), (Varga & Kozakiewicz, 2006)

have been carried out including chemical (Bellí, Marín, Sanchis, & Ramos, 2006), physical (Var, Kabak, & Erginkaya, 2008) and microbiological (Péteri, Téren, Vágvölgyi, & Varga, 2007) to look for ways to eliminate or minimize OTA contaminants from foods. However, the drawback of chemical and physical methods pose a challenge to be used effectively for the control of OTA-producing fungi. For instance, physical methods such as filtration or adsorption could reduce sensorial attributes such as color, taste, aroma and other desirable properties (Grazioli, Fumi, & Silva, 2006).

The use of synthetic chemicals can effectively control the growth and proliferation of OTA-producing fungi and contamination in food. However, the use of synthetic fungicides could lead to environmental pollution and development of resistance by toxigenic fungi and other major plant pathogens. Again, the use of synthetic chemicals have been noted to have cumulative and residual effects that are harmful to human life (Zhang, Wang, Zheng, & Dong, 2007). Thus, under the current circumstances, BCAs remain the only promising and safe method to control OTA and other plant pathogens without harmful effect to the environment, human and development of resistance by pathogens.

Accordingly, there has been increasing interest, by researchers during the last decade to employ biological methods to control OTA in foods, by researching into yeast, bacteria and non-toxic fungi for their ability to inhibit the growth of OTA-producing fungi, as well as detoxify OTA through binding or degradation to limits acceptable by legislation (2–10 µg/L) (Bleve, Grieco, Cozzi, Logrieco, & Visconti, 2006). Moreover, as highlighted by Wilson and Wisniewski (1994), biological control with microbial antagonists proved to be a viable alternative either singly or in combination with other eco-friendly methods to minimize the use of synthetic fungicides. In choosing a microbial antagonist therefore, it is recommended to take a closer look at the points stated herein: (a) the level of disease control by the antagonist have to be high (95–98%), (b) the possibility of commercializing the antagonist in the market, (c) the food safety requirements regarding the usage of the microbial antagonist and (d) the market value of the fruit to justify the application or usage of the microbial antagonist (Chalutz & Droby, 1998). In line with these, several microbial antagonists have been investigated to determine their efficacy against numerous postharvest fungal pathogens such as *Aspergillus* spp., *penicillium* spp., *Botrytis* spp., *Monilia* spp., and *Rhizopus* spp (Droby et al., 2002; Zahavi et al., 2000). According to Chalutz and Droby (1998); Spadaro and Droby (2016), yeasts appear to have most of the qualities stated above, henceforth in the recent past, studies have been centered on the identification and selection of antagonist yeast strains. Indeed, no single BCA (Biological Control Agents involve the use of bioeffector methods to control pests, and plant diseases by employing microorganisms) method has proved to be very successful in the control of fruit rots and mycotoxin producing fungi (Mahunu, Zhang, Yang, Li, & Zheng, 2015).

In view of the above, there is the need for a concerted effort to harmonize and integrate a mixture of non-competitive and complementary BCAs, which are likely to have broad spectrum of postharvest disease control. The objectives of this review were to: (1) assess the removal of OTA from wine using microbiological methods. (2) recount the successes attained after the emergence of

microbial antagonists to control pre- and postharvest OTA-producing fungi in grape berry and the way forward, (3) assess the efficacies and commercial potentials of BCAs in the control of plant diseases and biodegradation of OTA.

2. OTA detoxification pathway

OTA is a 7-carboxy-5-chloro-8-hydroxy-3, 4-dihydro-3 R-methylisocoumarin, linked through its 7-carboxy group to L-β-phenylalanine by an amide bond (Van der Merwe, Steyn, & Fourie, 1965). OTA is very stable at high temperature thus, industrial processing of raw materials of feed and food containing OTA does not eliminate it and the toxin remains an integral part in the end-product. The bio-synthesis pathway of OTA has still not been totally explicated. Nevertheless, it is clear that the pathway encompasses some critical steps, such as the bio-synthesis of isocoumarin group by the catalyzing action of polyketide synthase (PKS), the catalyzing reaction by the enzyme peptide synthetase linked with the amino acid phenylalanine by the aid of the carboxyl group, and the chlorination step.

However, the order of these reactions are not yet well-defined. It has been reported that carboxypeptidase A activity enzymes have the ability to degrade OTA (Amézqueta, González-Peñas, Murillo-Arbizu, & de Cerain, 2009) (Fig. 1) though there are some toxicity challenges regarding the use of enzymes to degrade OTA in produce because of their undesirable effects on non-targeted microbes on the quality of wine. Some bacteria (*Streptococcus*, *Bacillus* and *Bifidobacterium*) and fungi (*Alternaria*, *Penicillium* and *Botrytis*) have been revealed to be able to degrade OTA (Petruzzi, Sinigaglia, Corbo, Campaniello, Speranza, & Bevilacqua, 2014). Its production to a very large extent depends on intrinsic factors such as the nutritional composition, the type of food matrix, water activity, plant type and moisture content.

3. Preharvest application of BCAs

Generally, microbial control agents can be applied either at preharvest or postharvest depending on which method is effective in controlling or suppressing the pathogen (OTA producing fungi) (Sharma, Singh, & Singh, 2009). Nonetheless, preharvest application of BCAs allows for a better colonization of the fruit wound and surface thus, protecting the fruits during storage (Ippolito & Nigro, 2000). BCAs encounter variable and harsh environmental conditions on the field and these threaten their survival than those conditions encountered during storage (Ippolito & Nigro, 2000). This reduces the number of microbial control agents that are suitable for preharvest application.

For preharvest application to be successful, the microbial antagonist should possess the ability to anchor or attach itself to the fruit surface, as persistent attachment by the antagonist offers a better colonization and prevents wind or rain from detaching or dislodging it (Dickinson, 1986). For instance, as a way of attaching itself, the yeast-like fungi (*Aureobasidium pullulans*) produce slime, predominantly made of extracellular polysaccharides on the phylloplane which aids its adhesion (Meng & Tian, 2009). It is well

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