Chapter 8
Confrontation of Microbes with Mycotoxin-Producing Strains

Ye Tian and Aibo Wu

Abstract Mycotoxins, as secondary microbial metabolites, frequently contaminate cereal grains and pose a serious threat to human and animal health at the global levels. Except for physical separation and chemical treatments, biological control with functional agents has been proved to more realistically manage mycotoxin contaminations, especially from the view of the whole food chain. In general, functional biological control agents (BCAs) cover the scope of antagonistic microbes, natural fungicides derived from plants, and detoxification enzymes. In this chapter, we summarize the developed BCAs against various agro-important mycotoxins (DON, ZEN, FB1, AFB1, etc.) on cereal grains and fruits, with more emphasis on its significance on the inhibition or degradation of mycotoxin contaminations, concerning food security and food safety.

Keywords Biological control agents (BCAs) · Degradation · Detoxification · Contamination mycotoxins

8.1 Introduction

It is well-known that some plant pathogens are responsible for crop diseases and mycotoxin contaminations in the whole food and feed production chain. Trichothecenes, zearalenone, and fumonisins are the major mycotoxins produced by various *Fusarium* species collected in different regions (Bertero et al. 2018). In the past several years, a few of lab or field experiments investigated the potential of beneficial microbes to manage plant diseases. The diseases caused by various species of toxigenic fungi not only cause yield losses of cereal grains but also lead to mycotoxin contamination, posing a great risk to the health of humans and animals. The use of the traditional chemical fungicides to manage pathogenic fungi is

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effective, but it may bring some adverse effects, such as environment pollution, causing fungal genetic variation, which is not good for long-term use (Yuen and Schoneweis 2007). Meanwhile, biological control of plant diseases caused by toxins with beneficial microbes is an emerging alternative method, which is environment-friendly and fit the requirements of sustainable agricultural development (Alberts et al. 2016).

Here, we will discuss new well-studied biological control agents (BCAs) against toxigenic fungi and mycotoxin contamination (Table 8.1). We will focus on various antagonistic actions of the BCAs on pathogenic fungi growth and their ability to inhibit mycotoxin production, which would be beneficial to have more understanding on the antagonistic potentials of BCAs on mycotoxin contamination with respect to the theme of food safety.

The well-studied antagonists mainly consist of *Trichoderma*, *Clonostachys rosea* (Schoneberg et al. 2015), *Cladosporium cladosporioides*, *Aureobasidium pullulans*, *Bacillus* and *Pseudomonas* genera, and yeast (Tian et al. 2016a). These antagonistic microbes can be used directly for inhibition of growth and mycotoxin production of fungi in pre-harvest stage or applied on crop residuals to inhibit spore production after harvest.

8.2 Antagonistic Fungi

Of the abovementioned BCAs, *Trichoderma* genus has been widely investigated in both lab and field experiments, because *Trichoderma* is a nonpathogenic genus for crops, which could produce a series of antibiotics against plant pathogens (Mukherjee et al. 2013). In addition, they grow faster than competitors which could inhibit other fungal growth. Another important mechanism for managing toxicogenic fungi is mycoparasitism mediated by production of the cell wall-degrading enzymes including cellulases, chitinase, and glucanases (Vinale et al. 2008). Consequently, *Trichoderma* isolates are potential candidates to control pathogenic fungi. For instance, an antagonistic strain *T. gamsii* 6085 was tested on its potentials against *F. culmorum* and *F. graminearum* (Matarese et al. 2012). This *Trichoderma* strain could suppress DON production by the two *Fusarium* pathogens up to 92%. Another study indicated that a *Trichoderma* strain, T-22, was able to decrease the perithecia formation of *F. graminearum* by 70% in a field experiment (Inch et al. 2007). Moreover, it is also very important to understand details in the interplay between antagonistic *Trichoderma* and toxigenic *Fusarium*. Recently, we demonstrated the potentials of *Trichoderma* genus for control of deoxynivalenol (DON) and zearalenone (ZEN) producers by dual culture on PDA medium. Also, we investigated the metabolic activity of the *Trichoderma* isolates on DON and ZEN. The achieved data suggested that *Trichoderma* isolates were effective antagonists to manage the growth and mycotoxin production of the tested DON- or ZEN-producing

Table 8.1 The mentioned antagonistic microbes for control of mycotoxin contamination

	Functional BCAs	Mechanism	References
Antagonistic fungi	*Trichoderma* strains	Inhibiting sporulation, growth and/or mycotoxin	Schoneberg et al. (2015)
	Clonostachys rosea	production of pathogens, or	Schoneberg et al. (2015)
	Cladosporium cladosporioides	bio-transform mycotoxin into less toxic compounds	Schoneberg et al. (2015)
	Aureobasidium pullulans		Wachowska and Głowacka (2014)
	Trichoderma strains		Schoneberg et al. (2015)
	Trichoderma strains		Matarese et al. (2012)
	Trichoderma T-22		Inch et al. (2007)
	Trichoderma atroviride P1		Lutz et al. (2003)
	Trichoderma strains		Tian et al. (2016b)
	Trichoderma strains		Tian et al. (2018)
	Trichoderma strains		Ferrigo et al. (2014)
	Trichoderma strains		Mukherjee et al. (2012)
	Panax notoginseng		Zheng et al. (2017)
Antagonistic bacterial	*Bacillus subtilis* SG6		Zhao et al., (2014)
	Bacillus subtilis RC 218 and *Brevibacillus* sp. RC 263		Palazzini et al., (2016)
	Bacillus amyloliquefaciens		Shi et al. (2014)
	Shewanella algae strain YM8		Gong et al. (2015)
	P. fluorescens		Palumbo et al. (2007)
	Piriformospora indica		Mousa et al. (2015)
	Pseudomonas and *Bacillus* genera		Figueroa-López et al. (2016)
	B. amyloliquefaciens		Pereira et al. (2007)
Antagonistic yeast	*Cryptococcus*		Khan et al. (2004)
	Candida parapsilosis IP1698		Niknejad et al. (2012)
	Cryptococcus spp., *Kluyveromyces* sp,., and *Saccharomyces* spp.		El-Tarabily and Sivasithamparam (2006)

Fusarium strains, and results demonstrated that *Trichoderma* isolates could bio-transform DON into D3G via glycosylation (Tian et al. 2016b) and bio-transform ZEN into ZEN-S via sulfation (Fig. 8.1) (Tian et al. 2018). Interestingly, except for the obvious inhibition effects on the fungal growth of *F. graminearum* via confrontation, the masked form D3G appeared at various levels, highly co-related to the

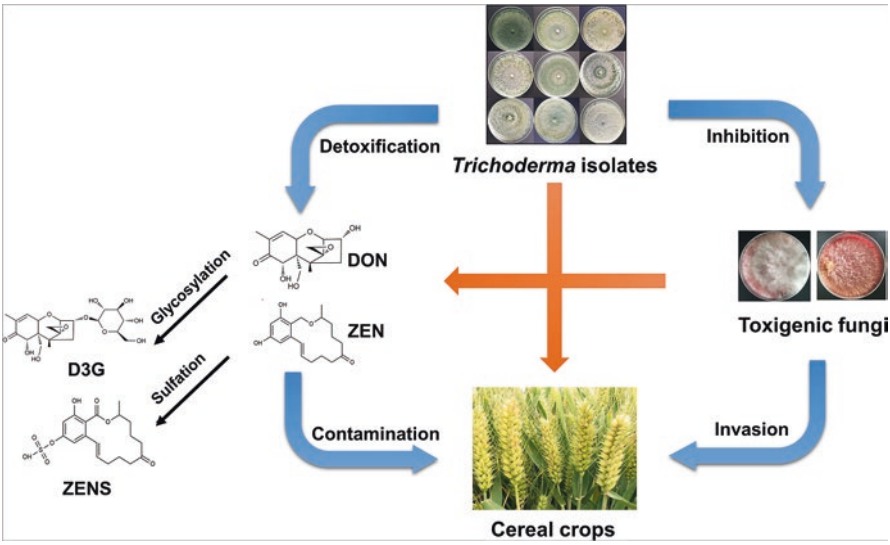

Fig. 8.1 *Trichoderma* isolates were effective BCAs to manage the growth and mycotoxin production of DON- or ZEN-producing fungi. In addition, we provided evidences that *Trichoderma* isolates were capable of bio-transforming DON into D3G via glycosylation and bio-transforming ZEN into ZEN-S via sulfation. (Tian et al. 2018)

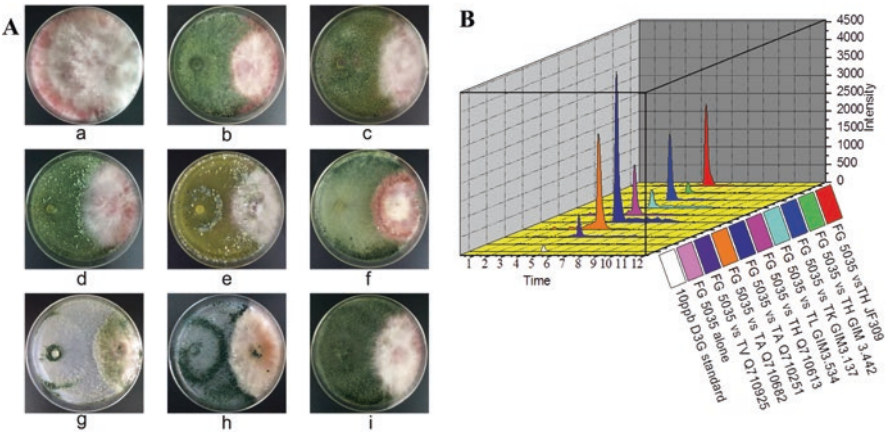

Fig. 8.2 Different trichoderma were co-cultured with *F. graminearum* 5035; (**a**) colony morphology of *F. graminearum* 5035 in dual-culture tests after incubation on the potato dextrose agar (PDA) medium; (**b**) the concentration of the D3G. (Tian et al. 2016a, b)

inhibition efficiencies of different *Trichoderma* isolates (Fig. 8.2). On the other side, some chitinase-encoding genes were upregulated in mycoparasitic *Trichoderma* spp. when confronted with *Fusarium*, while another study demonstrated that mycotoxin DON production could suppress one chitinase gene (nag1) expression in a *T.*

atroviride strain P1 as a negative signal in interaction of *Trichoderma* and *Fusarium* species (Lutz et al. 2003).

Furthermore, it has been proved that *T. harzianum* could promote plant growth and could enhance crop resistance against pathogenic fungi. Results of the dual culture of *Trichoderma* spp. with *F. verticillioides*, *F. graminearum*, and *A. flavus* showed that *T. harzianum* could inhibit the pathogen *F. verticillioides* in maize by inducing resistance though inducing signaling pathways (Ferrigo et al. 2014). A recent work identified some new endophytic *Trichoderma* strains capable of protecting plants against diseases by invading plant tissue and then inducing transcriptomic changes (Mukherjee et al. 2012). It is said that endophytic fungi could balance the system and promote host growth. Also, the endophytic fungi diversity of *Panax notoginseng* was investigated and then antagonistic potentials of endophytic fungi on phytopathogens causing root rot evaluated. Their results suggested that endophytic fungi would be a source for screening new natural compounds for biocontrol of plant root rot disease (Zheng et al. 2017).

8.3 Antagonistic Bacterial

The nonpathogenic bacteria are also being widely studied as antagonists against plant diseases recently (Shi et al. 2014). Antagonistic bacterial strains are usually endophytes which inhabit the rhizosphere or anthers of crops, which could not cause adverse effects on their host. For example, a *Bacillus subtilis* strain SG6 from anthers of wheat was proved that it could inhibit the mycelial growth, sporulation of conidia, and DON production of *F. graminearum* (Zhao et al. 2014). In addition, two bacterial strains of *B. subtilis* RC 218 and *Brevibacillus* RC 263 from wheat anthers could remarkably decrease the incidence of FHB diseases and mycotoxin DON contamination (Palazzini et al. 2016). This research work was done in semi-controlled field conditions. In another study, *B. amyloliquefaciens* isolated from peanut shells exhibited strong inhibitory effects on the mycelium growth and DON production of *F. graminearum* (Shi et al. 2014). Interestingly, a strain YM8 of *Shewanella algae* isolated from sediment, producing volatile organic compounds with inhibition effects against nine different important plant pathogens. This research work which indicates that the bacteria from marine is a potential and promising resource for screening effective BCAs against the growth and mycotoxin production of plant pathogens (Gong et al. 2015). Another work reported that *P. fluorescens* could produce antifungal compounds and chitinase, which had inhibitory effects on *A. flavus* and *F. verticillioides* growth (Palumbo et al. 2007).

Recently, it has been reported that *Piriformospora indica* was able to reduce both the severity the crop disease caused by *F. graminearum* and extended by DON contamination (Rabiey and Shaw 2016). In addition, some novel endophytes (*Piriformospora indica*) were predicted that they were able to detoxify DON in vitro, but the performance of the strains has not verified under field conditions (Mousa et al. 2015).

The rhizobacterial *Pseudomonas* and *Bacillus* genera could significantly inhibit the mycotoxin produced by *F. verticillioides* up to 70% (Figueroa-López et al. 2016). In another study, the authors treated the seed with B. *amyloliquefaciens*, and the amount of fumonisins was reduced in field trails (Pereira et al. 2007). Next, the results were confirmed in a 2-year field trials with the same *B. amyloliquefaciens* (Pereira et al. 2007).

8.4 Antagonistic Yeast

Besides the antagonistic fungal strains and bacteria, yeast are also promising candidates for mycotoxin control. It was reported that the yeast *Cryptococcus* spp. could control plant FHB disease by 50–60% on susceptible wheat in field tests (Khan et al. 2004). The yeast *Candidaparapsilosis* IP1698 could inhibit aflatoxin production up to 90% at various conditions of pH and temperatures (Niknejad et al. 2012). Yeasts such as *Cryptococcus* spp., *Kluyveromyces* spp., and *Saccharomyces* spp. were reported that they could produce bioactive metabolites targeting various pathogens for management of their growth (El-Tarabily and Sivasithamparam 2006).

8.5 Conclusion

Managing toxicogenic fungi and mycotoxin contamination in both pre-harvest and post-harvest stages is very important for ensuring food safety (Wegulo et al. 2015). It is still challenging to find stable and efficient BCAs to control the growth of phytopathogenic fungi and mycotoxin production in different stages of food production and storage. We know that a pathogenic fungus could produce different mycotoxins because of the biosynthetic pathways, and the next research work should focus on the management of multi-mycotoxin contamination with effective BCAs. Consequently, it will be more practical to select a biocontrol agent which is capable of suppressing the production of different mycotoxins at the same time. Another point is that the BCAs should be much more tolerant of different mycotoxins, which would guarantee efficiency when confronted with different toxicogenic fungi.

Though several BCAs have been well investigated on their activities against various toxigenic fungi in the lab experiments, their potentials on toxigenic fungi against pathogens in field experiments have not been further validated in depth. The performance of BCAs for managing pathogens in vivo or in field trails might be diffident, because there are more related factors affecting BCA's performance in field conditions, such as the nutrient differences in soil and the different microbial community. Other important factors which may affect the activities of BCA are the delivery method of BCAs to the crops, the delivery form of BCAs, and the time and route to apply BCAs against plant pathogens. Consequently, it is important to consider all the different factors in field trails which may affect the antagonistic results,

and field experiments should be comprehensively carried out to assess BCA's potentials against toxigenic fungi.

Future work needs to be done to comprehensively elucidate the biological control mechanisms and there are few novel enzymes responsible for mycotoxin transformation, both RNA-seq analysis for identification of key genes and metabolomic analysis by HRMS for screening unknown mycotoxin metabolism need to be done. In total, investigations on the interaction between BCAs and toxicogenic fungi, especially the inside mechanisms of BCAs on mycotoxin production of fungi, which will provide more new insights on applicable biocontrol practices in food safety and agricultural protection.

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