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Review: Prevention and Reduction of Mycotoxin by Antagonistic Microorganism

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Abstract

Mycotoxin is widely known as one cause of foodborne disease, produced by toxigenic fungi. Any country should be aware about this high risk potency by knowing the mycotoxin, affected commodities, fungal sources, and toxicity effect to human or animal. Controlling mycotoxin could be done by physic, chemical, and biological methods. The microbial characteristic used for biological agent should be evaluated including the inability to produce toxic substance, tendency to multiply, colonize, survive, safety, and applicability to the environment. Studies related to mycotoxin biocontrol by using antagonistic microorganism can be focused on (1) the effect to the mycotoxin, (2) the growth of microorganism, or (3) the application to food both raw material and processed products. Consideration to combine more than one species of microorganism instead of a single species also has been taken to achieve more effective result. For example, S. cerevisiae has been used together with LAB to control certain mycotoxin. Further studies are needed to develop the possibility of other biological agents and the effect of their application, which in the next have the potency as manufacturing products.

Keywords: antagonistic microorganism, biological agent, combination, microbial characteristic, mycotoxin biocontrol, potency

Introduction

Mycotoxin prevalence becomes higher with the availability of supporting conditions such as proper climatic changes, plentiful substrates, and lack of controls. In general, food and feed are very diverse in microbial growth substrates, due to variation in: intrinsic factors such as nutrients, pH, reduction-oxidation potential, water activity, antimicrobial components, and biological structure; and also extrinsic factors such as storage temperature, equilibrium relative humidity, and environmental gases. Besides controlling through regulations, many studies of controlling mycotoxin have also been done throughout the world. The using of antagonistic microorganism or metabolite compounds produced by microorganism as biological agent has many advantages, such as mild reaction conditions, target specificity, efficiency and environmental friendly.

Principle of Biocontrol by using Antagonistic Microorganism

Biological control or biocontrol can be defined as: i) a method of managing pests by using natural enemies, ii) an ecological method designed by man to lower a pest or parasite population to acceptable sub-clinical densities, iii) to keep parasite populations at a non-harmful level using natural living antagonists (Baker, 1987). Biological control agents act against the pathogen through different modes of action. Antagonistic interactions that can lead to biological control include antibiosis, competition and hyperparasitism (Bloom et al., 2003; Bull et al., 2002).

Antibiosis occurs when antibiotics or toxic metabolites produced by one microorganism have direct inhibitory effect on another. Competition occurs when two or more microorganisms require the same resources in excess of their supply. These resources can include living space, nutrients, and oxygen. In a biological control system, the more efficient competitor, i.e., the biological control agent out-competes the less efficient one, i.e., the pathogen. Hyperparasitism or predation results from biotrophic or necrotrophic interactions that lead to parasitism of the pathogen by the biological control agent (Bloom et al., 2003; Bull et al., 2002).

Increasing need of crop protection from toxigenic fungi attacks have forced scientist as well as biological and chemical industries to develop a mass production of biological control agent. Sharma et al. (2009) showed some biocontrol products have been developed to control postharvest diseases and mycotoxins (Table 1).

Table 1. Biocontrol products developed for control of postharvest diseases and mycotoxins (Sharma *et al.*, 2009)

Product	Microbial agent	Food commodity	Manufacturer/distributor
AF36	*Aspergillus flavus* AF36	Corn and cotton	Arizona Cotton Research and Protection Council, USA
Afla-guad	*A. flavus* strain NRRL21882	Peanuts and corn	Syngenta Crop Protection, USA
AQ-10 biofungicide	*Ampelomyces quisquolis* Cesati ex Schlechtendahl	Apples, grapes, strawberries, tomatoes, and cucurbits	Ecogen, Inc., USA
Aspire	*Candida oleophila* strain 1-182	Apple, pear, citrus, cherries, and potatoes	Ecogen, Inc., USA
Biosave 10LP, 110	*Pseudomonas syringae* (strain 10 LP, 110)	Apple, pear, strawberries, and potatoes	Eco Science Corporation, USA
Blight Ban A 506	*Pseudomonas fluorescence* A506	Onion	Nu Farm, Inc., USA
Contains WG, Intercept WG	*Coniothyrium minitans* Campbell	Vegetables	Prohyta Biologischer, Germany
Messenger	*Erwinia amylovora* (Burrill) Winslow *et al.*	Vegetables	EDEN Bioscience Corporation, USA
Rhio-plus	*Bacillus subtilis* FZB 24	Potatoes and other vegetables	KFZB Biotechnik, Germany
Serenade	*B. subtilis*	Apple, pear, grapes, and vegetables	Agro Quess, Inc., USA

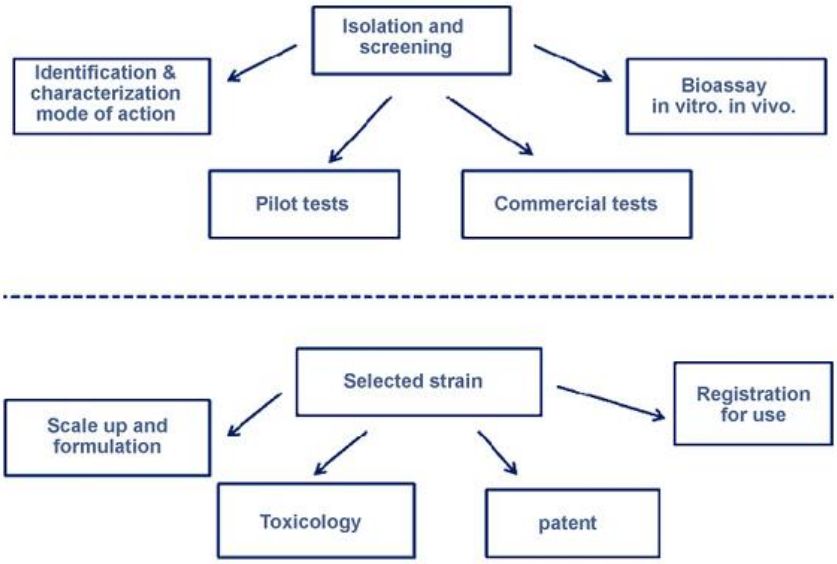

Figure 1. Factors involved in the development of a biocontrol product (Droby *et al.*, 2009)

Droby et al. (2009) extended information about characteristics of an ideal postharvest antagonist for commercial development, such as: genetically stable, effective at low concentration, not fastidious in its nutrient requirements, able to survive adverse environmental conditions, effective against a wide range of pathogens on different commodities, amenable to production on inexpensive growth media, amenable to formulation with a long shelf-life, easy to dispense, resistant to chemicals used in the postharvest environment, not detrimental to human health, and compatible with commercial processing procedures. Scientist should consider the factors involved in the development of a biocontrol product (Figure 1). Among the important factors to consider are: (1) biosafety of the selected antagonist, (2) patent potential, (3) growth requirements and shelf life, (4) range of activity (commodities and pathogens), and (5) ease of use. If any of these factors are of concern, one may want to abandon further development despite the efficacy of the selected antagonist.

Studies of Antagonistic Microorganism for Mycotoxin Biocontrol

Utilization of microorganism by using their enzymatic metabolites to detoxify mycotoxins in food and feed has advantages such as mild reaction conditions, target specificity, efficiency and environmental friendly. The microorganism must be evaluated for a set of selection criteria for further use in biocontrol field experiments. Inability to produce toxic substances for biological systems and propensity to multiply, colonize and survive are the most important selection criteria to make sure that the selected antagonistic microorganism strains are safe and applicable when they introduced into the environment. The biocontrol of mycotoxin which is discussed in this review are including aflatoxin (AF), ochratoxin A (OTA), patulin (PAT), and fumonisin biocontrol.

1. Aflatoxin biocontrol

Aflatoxins is a group of mycotoxins which is mainly produced by certain strains of Aspergillus flavus and A. parasiticus. Its association with various diseases, occurrence

which is caused by several factors and its potency of carcinogenic effect as well as its acute toxicological effect bring this mycotoxin to be probably the famous and the most intensively studied mycotoxin throughout the world. Aflatoxin can be naturally found in some foods including grains or cereals, spices, nuts, dried fruit, even in processed food such as breakfast cereals.

The effect of Saccharomyces cerevisiae RC008 and RC016 strains, previously selected based on their AFB1 mycotoxin binding ability and beneficial properties, against Aspergillus carbonarius and Fusarium graminearum under different interacting environmental conditions was evaluated by Armando et al. (2013). In vitro studies on the lag phase, growth rate and ochratoxin A/zearalenone and DON production were carried out under different regimens of aW (0.95 and 0.99); pH (4 and 6); temperature (25 and 37 °C) and oxygen availability (normal and reduced). Both yeast strains showed antagonistic activity and decreasing growth rate compared to the control. In general, the RC016 strain showed the greatest inhibitory activity. Except at the interacting condition 0.95 aW, normal oxygen availability and 37 °C, at the both pH values, A. carbonarius and F. graminearum were able to produce large amounts of mycotoxins in vitro. In general, a significant decrease in levels of mycotoxins in comparison with the control was observed. S. cerevisiae RC008 and RC016 could be considered as effective agents to reduce growth and OTA, ZEA and DON production at different interacting environmental conditions, related to those found in stored feedstuff. The beneficial and biocontrol properties of these strains are important in their use as novel additives for the control of mycotoxigenic fungi in stored feedstuffs.

Bacteria and the metabolites have been widely introduced to be biocontrol agent against aflatoxigenic fungi; even it has been developed into commercial product. Bacillus subtilis, for example, was first introduced as an inhibitor of growth and AF production of aflatoxigenic fungi and the effective compound, iturin A, had been patented for the control of AF in nuts and cereals (Kimura & Hirano, 1988). A strain of marine Bacillus megaterium had been studied by Kong et al. (2010) as a biocontrol of A. flavus on peanut kernels. The degradation of another type of aflatoxins, Aflatoxin B1 (AFB1) by Bacillus subtilis UTB SP1 isolated from pistachio nuts had been investigated by Farzaneh et al. (2012).

Shams-Ghahfarokhi et al. (2013) suggested terrestrial bacteria from agricultural soils as versatile weapons against aflatoxigenic fungi. There are some important limitations from the type of vegetative compatibility groups which shows the progeny of the fungus for AF-producing ability to geographic limitations in selection of atoxigenic strains. Considerable tolerance of B. subtilis and P. chlororaphis to environmental stresses, their large capacity for producing diverse array of beneficial antifungal metabolites and their readily producing by current fermentation technology make them promising tools for biocontrol of aflatoxigenic fungi in practice. Bacterial population from the genera Bacillus and Pseudomonas identified in pistachio, maize and peanut fields in the present study with potent antagonistic activity against aflatoxigenic Aspergillus parasiticus can potentially be developed into new biocontrol agents for combating AF contamination of crops in the field.

Examples of aflatoxin biocontrol agent are Afla-Guard and AF36. This product consists of nontoxigenic strains of A. flavus which does not produce aflatoxin. The nontoxigenic strain of A. flavus was

investigated for its biocontrol activity against the toxigenic strains. Selection of A. flavus isolates was made for biological control of aflatoxins in corn. Dorner (2009) applied nontoxigenic A. flavus to control AF contamination in corn. Abbas et al. (2011) studied three non-aflatoxigenic strains of A. flavus which are A. flavus strain NRRL 21882 (Afla-Guard), K49, and AF36 compared in side by side field trials to evaluate their safety and efficacy in controlling both aflatoxin and cyclopiazonic acid (CPA) in corn. The result showed that A. flavus strain NRRL 21882 and K49 did not produce CPA, whereas AF36 controls the production of aflatoxins, but also produces CPA, which is a concern for the safety of crop products to be used livestock feed and human food. The effect in other crop has been studied by Zanon et al. (2013) who suggested the using the strategy of competitive exclusion A. flavus AFCHG2 can be applied to reduce aflatoxin contamination in Argentinean peanuts.

Corassin et al. (2013) studied application of yeast combined with bacteria to bind aflatoxin M1 (AFM1) in UHT (ultra-high-temperature) milk. They evaluated the ability of a S. cerevisiae and three LAB strains (Lactobacillus rhamnosus, Lactobacillus delbrueckii spp. bulgaricus and Bifidobacterium lactis), alone or in combination, to bind aflatoxin M1 (AFM1) in UHT skim milk spiked with 0.5 ng AFM1 mL-1. All the LAB pool (1010 cells mL-1) and S. cerevisiae (109cells mL-1) cells were heat-killed (100°C, 1 h) and then used for checking the effect of contact time (30 or 60 min) on toxin binding in skim milk at 37°C. The mean percentages of AFM1 bound by the LAB pool in milk were 11.5±2.3 and 11.7±4.4 % for 30 and 60 minutes. Compared to the LAB pool, S. cerevisiae cells had higher capability to bind AFM1 in milk (90.3±0.3 and 92.7±0.7% for 30 and 60 min, respectively), although there were not significant differences between the contact times evaluated. S. cerevisiae and LAB pool, a significant increase was observed in the percentage of AFM1 bound (100.0%) during 60 minutes. It is apparent that bacterial viability is not a prerequisite for removal of AFM1 by LAB. Non-viable cells of S. cerevisiae and LAB strains may be useful for completely removing AFM1 from milk containing up to 0.5 ng mL-1, without any changes in the flavor or acidity of milk caused by fermentation. Additional studies are needed to investigate the mechanisms involved in the removal process of toxin by S. cerevisiae and/or LAB and the factors that affect the stability of the toxin sequestration aiming the commercial application in the dairy industry.

2. OTA biocontrol

Produced by several species of Aspergillus and Penicillium, Ochratoxin A (OTA) as the most economically important ochratoxin type is the most toxic form of ochratoxin and commonly found in several kinds of crop. OTA contamination generally occurred in cereal grains, grapes, coffee, tree nuts, and figs. Microorganisms which have been used for OTA biocontrol study can be categorized into yeast and bacteria. In the yeast group, there are some species which has been known to have activity in controlling OTA such as Trichosporon mycotoxinivorans, Aureobasidium pullulans, Debaryomyces hansenii, Saccharomyces cerevisiae, Kluyveromyces thermotolerans, and Metschnikowia pulcherrima.

Trichosporon mycotoxinivorans is yeast which has been developed as a commercial product for detoxifying OTA in animal feed (Molnar et al. 2004). In the other research, this species also presented the ability to decarboxylate ZEA (Molnar et al. 2004; Vekiru et al. 2010). Aureobasidium pullulans was

used as a biocontrol agent in wine, preventing OTA accumulation in grapes and decreasing aspergillosis symptoms (De Felice et al. 2008). Reduction of OTA produced by Aspergillus westerdijkiae by using Debaryomyces hansenii CYC 1244 had been studied by Gil-serna et al. (2011). S. cerevisiae also has been promoted as commercial product in case of mycotoxin biocontrol. Velmourougane et al. (2011) inoculated S. cerevisiae to manage A. ochraceus and OTA contamination in coffee during on-farm processing. Ponsone et al. (2011) applied biocontrol as a strategy to reduce the impact of OTA and Aspergillus section Nigri in grapes by using Kluyveromyces thermotolerans in preventing the growth and OTA accumulation both in vitro and in situ. Environmental factors also need to be studied especially about the affection towards the activity of biocontrol agents against ochratoxigenic Aspergillus carbonarius on wine grape (De Curtis et al. 2012).

In the group of bacteria, detoxification of OTA was done by Fuchs et al. (2008). The study used lactic acid bacteria (LAB) such as Lactobacilli, Bifidobacteria, and Streptococci to control OTA as well as PAT. The findings showed that Lactobacillus acidophilus VM 20 caused a decrease of OTA by ≥95%. Comparison between bacteria and yeast performed as composites also had been studied by Kapetanakou et al. (2012) to inhibit Aspergillus carbonarius growth and reduce OTA. Composites of 16 yeasts (16YM) and 29 bacterial (29BM) strains (107 cfu/mL) to estimate the kinetics of OTA reduction at 25°C for 5 days. The yeast composites were YM1 (Hanseniaspora guilliermondii, Kluyveromyces dobzhankii, Pichia fermentas, Issatchekia occidentalis), YM2 (Metschnikowia pulcherrima, Hanseniaspora uvarum, Issatchenkia terricola, Zygosaccharomyces bailii), YM3 (Zygosaccharomyces bailii, Kazachstania hellenica, Hanseniaspora opuntiae), YM4 (Saccharomyces cerevisiae, Pichia guilliermondii, Lachencea thermotolerans, Issatchenkia orientalis), and 16YM (all of the yeast strains). The result showed that none of the bacteria composites inhibited A. carbonarius growth. However, the high inoculum of yeast composites (105 cfu/mL) showed more efficient fungal inhibition compared to cell density of 102 cfu/mL. All yeast composites showed higher OTA reduction (up to 65%) compared to bacteria (2–25%), at all studied assays.

3. Patulin biocontrol

Patulin (PAT) is mycotoxins mainly produced by certain species of Penicillium, Aspergillus, and Byssochylamys. PAT is able to contaminate several varieties of foods such as fruits, grains, and cheese. The possibility of PAT occurrence in raw food, manufacturing processes, or consumption practices can emerge food safety concern. Similar to the other mycotoxins, PAT biocontrol involves yeast and bacteria to cope toxigenic fungi growth and its produced PAT.

Biocontrol activity of yeast against both Penicillium expansum contamination and PAT production had been studied by Tolaini et al. (2010) by applying Cryptococcus laurentii in apple fruits. A modification was applied to achieve the effective result of biocontrol activity by using Lentinula edodes in order to enhance the biocontrol activity of Cryptococcus laurentii. Lima et al. (2011) studied integrated control of blue mould using new fungicides and biocontrol yeasts lowers levels of fungicide residues and PAT contamination in apples. Another yeast strain, a new strain of Metschnikowia fructicola, was studied by Spadaro et al. (2013) for controlling Penicillium expansum as well as its PAT accumulation on four cultivars of apple.

Application of yeast as antagonistic microorganism in apples by using Pichia caribbica to control blue mold rot and PAT degradation had been studied by Cao et al. (2013). The decay incidence of the blue mold of apples treated by P. caribbica was significantly reduced compared with the control samples. The higher the concentration of P. caribbica, the better the efficacy of the biocontrol. Germination of spores and growth of P. expansum were markedly inhibited by P. caribbica in in vitro testing. After incubation with P. caribbica at 20°C for 15 days, PAT produced by P. expansum in apples was significantly reduced, compared to the control. In vitro testing indicated that P. caribbica can degrade PAT directly.

Fuchs et al. (2008) used lactic acid bacteria (LAB) such as Lactobacilli, Bifidobacteria, and Streptococci to control PAT as well as OTA. The findings showed that Bifidobacterium animalis VM 12 reduced PAT levels by 80%. To proof that the decrease of the toxins by LAB from liquid medium results in a reduction of their toxic properties, micronucleus (MCN) assays were conducted with a human derived hepatoma cell line (HepG2). Indeed, a substantial decrease (39–59%) of OTA and PAT induced MCN formation was observed with the most effective strains detected in the chemical analyses. Furthermore, also the inhibition of the cell division rates by the toxins was significantly reduced. These findings indicate that certain LAB strains are able to detoxify the two toxins and may be useful to protect humans and/or animals against the adverse health effects of these compounds.

4. Fumonisin biocontrol

Fumonisins are produced primarily by several species of Fusarium which widely spread and contaminate several crops, mainly maize. Fumonisin B1 (FB1) is the most common and economically important form of fumonisin. Maize is the most commonly crop contaminated by fumonisins.

Affection of yeast to fumonisin biosynthesis of F. verticillioides had been studied by Dalié et al. (2012) by implementing Pediococcus pentosaceus strain L006 and its metabolites. Some extracellular metabolites produced in MRS medium by the P. pentosaceus strain L006 were able to significantly reduce fumonisin production in liquid medium as well as on maize kernels. Fumonisin yields by F. verticillioides inoculated on autoclaved maize kernels were reduced by 75 to 80% after 20 days of incubation. Further similar studies should be aimed that the use of an antagonistic bacterial agent is not only to manage fumonisin contamination, but also emphasizing the potential use of bacterial metabolites to counteract fumonisin accumulation in kernels.

Application of yeast as biological control agent against Fusarium and its mycotoxin also has been widely studied. To control Fusarium molds growth as well as its FB1 production in maize seeds, Fareid (2011) used Saccharomyces cerevisiae as a biocontrol agent. The result showed that the growth of Fusarium moniliforme was negatively correlated with different doses of S. cerevisiae while detoxification was positively correlated with the doses. This means that by addition of S. cerevisiae, the growth of F. moniliforme was significantly decreased and the percentage of FB1 detoxification was significantly increased gradually.

Application of bacteria to control fumonisins in maize has showed positive effect. Bacillus amyloliquefaciens and Enterobacter hormaechei were used as bacterial agents in reducing the infection pf Fusarium verticilloides and FB1 contents (Pereira et al. 2010). The findings showed that the number of colony forming units of F.

verticillioides obtained from harvested maize kernels was positively correlated with fumonisin B1 content. Therefore, the scientist also studied the impact of Microbacterium oleovorans and B. amyloliquefaciens as antagonist to F. verticilloides on maize seedlings growth and antioxidative enzymatic activities (Pereira et al. 2011).

Mechanism of Biocontrol

From the above studies, the mechanism of mycotoxin biocontrol occurred through: (i) microbial growth inhibition, (ii) mycotoxin binding, (iii) mycotoxin detoxification, (iv) mycotoxin adsorption, and (v) enzymatic degradation.

The restrictiveness of living spaces as well as substrate nutrients is suggested as the cause of microbial growth inhibition through competition. Other microorganisms show the antagonistic activity by inhibiting fungal spore germination. Degradation of aflatoxin content also has been investigated as the effect of its nontoxigenic strains growth, even the other toxigenic strains. These strains develop mycelium until it is ready to reach lysis. Scientist agreed the possibility of aflatoxin damaging components which are produced by the leakage. Investigation of OTA degradation as well as inhibiting its fungal strains leads to positive result. Antagonistic microorganism has ability to inhibit the growth of A. ochraseus mycelium, the forming of OTA, or to metabolize OTA into nontoxigenic form.

Aflatoxin binding by bacteria generally existed in physical properties and was hypothesized by the occurrence of a physical union to the cell wall components (polysaccharides and peptidoglycans) instead of a covalent binding or degradation by the microorganism metabolism (Corassin et al. 2013). Fuchs et al. (2008) concluded that subsequently experiments showed that the binding of PAT and OTA compounds depends on different parameters, i.e. the concentration of toxins, the cell density, the pH-value and the viability of the bacteria.

Detoxification is defined as any treatments working against toxins in food or feed in order to lowering the content. Detoxification process can be categorized into three different ways, which is by physical, chemical, and biological treatments. In the real applications, it could be more than one method of detoxification treatments which are used for controlling fungal and mycotoxin contamination. One of biological detoxification is by appearing fermentation process. Studies show the ability of antagonistic microorganisms to perform fermentation process in order to decrease certain mycotoxins content.

One of the strategies for reducing the exposure to mycotoxins is to decrease their bioavailability by including various mycotoxin adsorbing agents in food or feed, which leads to a reduction of mycotoxin uptake as well as distribution to the blood and the target organs. Guo et al. (2013) suggested that mycotoxin adsorption by biosorbent microorganism such as yeast generally has lower cost than clarification, filtration, or chemical addition. The biosorbent microorganism is supported by the proper pretreatment for the yeast and the acidity (pH) of the environment.

Gil-serna et al. (2011) studied OTA biosynthesis which was evaluated by quantification of expression levels of pks (polyketide synthase) and p450-B03 (cytochrome p450 monooxygenase) genes using newly developed and specific real time RT-PCR protocols. Both genes showed significant lower levels in presence of D. hansenii CYC 1244 suggesting an effect on regulation of OTA biosynthesis at transcriptional level. High levels of removal of extracellular OTA were observed by

adsorption to yeast cell walls, particularly at low pH (98% at pH 3). Another research about PAT degradation was studied by Guo et al. (2013), focusing on the biosorption of PAT from apple juice by caustic treated cider yeast biomass. The results showed that the adsorption percentage of PAT increased simultaneously with the increase of pH and approached equilibrium at pH 4.5. The removal of PAT increased with decreasing of toxin levels. The experimental data were analyzed using the Langmuir and Freundlich equations. The Langmuir constants were qmax (mg/g) = 8.1766 and b (L/mg) = 0.0640. In packed bed column studies, it was found that Ca-alginate gel was a good biosorbent for PAT removal and immobilized caustic treated yeast particles in the gel increased the biosorption capacity of the gel. Pizzolitto et al. (2012) analyzed the removal of FB1 by microorganism in co-occurrence with aflatoxin B1 and the nature of the adsorption process. The team evaluated the ability of S. cerevisiae CECT 1891 and L. acidophilus 24 to remove fumonisin B1 (FB1) from liquid medium. The removal process was proven to be fast and reversible which made the FB1 molecules was not experienced any chemical modification. The main thing which is required of FB1 removal is the structural integrity of microorganism's cell wall. The mechanism involved in the removal of FB1 is a physical adsorption (physisorption) of the toxin molecule to cell wall components of the microorganism. Studies of co-occurrence of both mycotoxins clearly showed that they did not compete for binding sites on the microorganism's cell wall and the presence of one toxin did not modify the efficiency of the organism in the removal of the other mycotoxin.

Some microorganisms have ability to produce cell wall-degrading enzymes that will eliminate the other competitor microorganism. One of the biocontrol roles of enzyme is as biotransforming or biodegradating agent. Biotransforming agents becomes another strategy performing the degradation of mycotoxins into non-toxic metabolites by using bacteria/fungi or enzymes. The other way is by blocking the biosynthesis way of mycotoxin. Enzymes are not always performing as catalisator in metabolic pathway. Researchers are carrying out to study the impact of enzymes from antagonistic microorganism to the mycotoxin production. During the growth of the antagonistic microorganisms, specific metabolites should be produced which affect the growth of mycotoxigenic fungi. Enzymatic reaction also occurred in fermentation process. Therefore, the implementation of yeast and bacteria, especially LAB, to control mycotoxin contamination can be linked to the role of fermentation. S. cerevisiae has been used in many researches of aflatoxin, OTA, ZEA, DON, and fumonisin biocontrol, whereas LAB has been applied in research of aflatoxin, PAT, and OTA biocontrol.

Conclusion

The presence of mycotoxin as a threat for food safety and security is considerably serious problem for human life. Produced by microorganism (fungi), mycotoxin fortunately can be controlled by implementing the role of other microorganism and or its metabolites. Studies have suggested antagonistic microorganism as a safe and efficient biocontrol agent to decrease mycotoxin content. The various modes of actions as well as the mechanisms make the efforts of mycotoxin prevention and reduction into many alternative ways. More developed scientific studies regarding these properties need to be conducted in order to select which agents are applicable and suggested to have high possibility in controlling mycotoxin.

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