

# Microorganisms involved in the decontamination of trichotecens, mycotoxins produced by *Fusarium* fungi

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## SUMMARY

Mycotoxins are toxic secondary metabolites synthesized by fungi, which can gravely affect human and animal's health. The methods for detoxification/decontamination of agricultural products contaminated with mycotoxins can be divided into physical, chemical and biological methods. Biological methods were often ignored or with few exceptions were only briefly treated in synthesis articles regarding mycotoxins detoxification/decontamination. This paper presents data concerning the decontamination of trichotecens realized by microorganisms.

Keywords: mycotoxins, lactic acid bacteria, yeasts, decontamination

Mycotoxins are toxic secondary metabolites synthesized by fungi, which can gravely affect human and animal's health (Rotter et al., 1996). The notion of secondary metabolite refers to those compounds resulted from the secondary metabolism of fungus, which are not necessary to the development of a microorganism, but are an important consequence of the growing process, forming in the final stages of the exponential growth phase of the fungus. These toxic secondary compounds, are known under the generic name of mycotoxins, and are considered to have appeared during the phylogenetic evolution process of micromycetes as one of their means of defence (Borker et al., 2001).

The „major” mycotoxins known for the severe intoxications they produce to human and animals are synthesized, mainly, by 5 important genres of fungi: *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Claviceps* (Sweeney and Dobson, 1998). The group of trichotecens includes approximately 60 biologically active molecules produced mainly by species of the genre *Fusarium* which contaminates the grains, mainly corn; trichotecens can be also

synthesized by species belonging to the genres *Myrothecium*, *Trichoderma*, *Trichothecium*, *Stachybotrys*.

Over 40 types of trichotecens were isolated and characterized in the years 1970s (Taranu et al., 2009), which in 1977 were classified in 4 groups according to the nature of the substitutes to the basic structure:

- **group A** is constituted from trichotecens which do not present the cetone function at position C8 and has as important representatives T-2 toxin (T2), diacetoxyscirpenol (DAS), and HT-2 toxin (HT-2);

- **group B** comprises trichotecens which have the cetone function at C8 and has as main representatives nivalenol (NIV), deoxynivalenol (DON), and X fusarenone;

- **group C** comprises trichotecens characterized by the presence of a supplementary epoxide group at position C7-8 and has crocotine as main representative;

- **group D** is constituted from trichotecens characterized by the presence of a macrocycle between C4 and C15 and has as important representatives verucarins, roridines and satratoxins (Taranu et al., 2009).

The methods for detoxification/decontamination of agricultural products contaminated with mycotoxins can be divided into physical, chemical and biological methods. Biological methods were often ignored (Riley et al., 1993) or with few exceptions (Bhatnagar et al., 1991) were only briefly treated in synthesis articles regarding mycotoxins detoxification/decontamination (Mueller, 1981; Doyle et al., 1982; Scott, 1991). This reflects the fact that a small number of studies on mycotoxins detoxification by biological methods compared with chemical or physical methods were made in the past.

The progress made in the field of biotechnology in the last years, as well as the fact that microbial populations can have unlimited catabolic capacities will probably determine in the not so far away future a modification of this tendency in favour of biological methods. Furthermore, EU Regulation (1831/2003) bans the use of chemical and thermal methods to decrease feed and food contaminants because they could alter their nutritive value. As opposed to the physical and chemical methods, biological detoxification is still incompletely defined. This way the fodder additives which bind mycotoxins, additives which improve the feed taste, and other nutritional interventions were grouped within the biological methods of decontamination (Charmley and Prelusky, 1994). Even mixing the contaminated grains with good quality grains to reduce the concentration of mycotoxins to acceptable levels is considered to be a biological method (Charmley and Prelusky, 1994; Charmley et al., 1995). The dilution of aflatoxins contaminated grains, called „criminal biological decontamination”, was largely used in the past (Patterson and Young, 1993). At present this practice is forbidden by the European

Community starting with January 1st 1999 (Directive of the European Commission 1525/98). According to Bhatnagar (1991), biological detoxification is defined as enzymatic degradation or transformation of toxins, which leads to the appearance of less toxic products. This classification includes mycotoxins and fycotoxins. This paper treats only the aspects related to the detoxification realized by microorganisms resident in the digestive tract, environment, etc.

The metabolic transformations of mycotoxins by the enzymes from animal tissues don't have practical applicability. The biological degradation of mycotoxins can be observed in the field and during the storage after harvest. Although great progress was made in identification of microorganisms, enzymes, and genes responsible for detoxification, the complexity of the systems responsible for degradation makes difficult the interpretation of observations, and prevents elucidating the underlying mechanisms. However, the obtained results can be used to develop some procedures for inactivation of mycotoxins in the complex conditions of agricultural systems, vegetal and animal production.

### **1. General information regarding the microbial degradation of mycotoxins**

Many feedstuffs, as well as dietary supplements, especially the ecologic ones could be contaminated with mycotoxins (Habeanu, 2010a, Habeanu, 2010b). Elimination of mycotoxins from the contaminated feed and food is an unsolved problem, and once the contamination is produced, there are only few strategies which can be adopted to limit the adverse effects in farm animals (Taranu et al., 2009).

Investigation of mycotoxins biodegradability became an area of great interest. Considerable efforts were made to isolate some microorganisms of different origins, which can detoxify mycotoxins *in vitro* and *in vivo* (Schatzmayr et al. 2006a).

Several microorganisms with mycotoxins degradation activity were isolated until now; the first was *Flavobacterium aurantiacum* with ability to detoxify aflatoxins (Ciegler et al. 1966). Wegst and Lingens (1983) provide evidence regarding the degradation of ochratoxin A by aerobic bacteria *Phenylobacterium immobile*. *Gliocladium roseum* detoxifies zearalenone by opening the ring structure with corresponding decarboxylation in a percentage between 80 and 90% (El-Sharkawy and Abul-Hajj, 1988). Recent data concerning the capacity of microorganisms to degrade trichotecens will be presented further.

### 1.1. Evidence of degradation in the field of trichotecens which contaminate grains

Indirect proofs indicate the reduction of trichotecens contamination of grains in the field, seeds left on the ground during winter, stored grains and compound feeds, although the degradation products were not identified. Scott et al., (1984) observed a rapid decrease of deoxynivalenol (DON) contamination in the field.

Miller and Young (1983, 1985) monitored DON content in the wheat artificially contaminated with *F. graminearum*. After an initial accumulation, they observed a decrease of DON concentration with 73%. These results suggested that DON degradation might be made by plants cells.

Regarding the stored grains, it was observed a continuous decrease of the concentration of DON, T-2, HT-2 and DAS after a period of 3-6 months of storage correlated with low temperature (4°C) (Karpainen et al., 1985). Indeed, Gilbert (1988) observed a decrease of DON concentration in contaminated corn stored at -18°C. The authors sterilized the grains by irradiation and they observed that at the same temperature, DON concentration remained the same; the sterilization by irradiation made DON reduction impossible. The author attributed this process to the disappearance of fungi remaining on the grains seeds. In a following study, all the samples of grains contaminated with DON were irradiated, and a decrease of DON concentration at all storage temperatures it was observed, except for the temperature of -18°C (Gilbert, 1995). DON disappearance from grains during the winter period was attributed to the degradation (Langseth et al., 1997). The apparent degradation of DON and other trichotecens in stored grains was frequently reported even at positive temperatures (Kallela et al., 1991; Langseth and Stabbetorp, 1996). However, Beattie et al., (1998) didn't find any modification in DON concentration in barley after storage in different conditions for a period of 7 months. The possibility of removing the toxin through washing wasn't considered, although DON is soluble in water.

It is easy to consider that the level of mycotoxins from natural and agricultural ecosystems is the result of competitive synthesis, transport, conjugation, liberation from bound forms and degradation. Anyway, the interpretation of the decrease in mycotoxins concentration in the natural context is difficult. Moreover, mycotoxins can be missed by chemical analysis due to their presence in conjugation forms, which can be made by the producing fungi (Chakrabarti and Ghosal, 1986), by other microorganisms (Kamimura, 1986) or by host plants (Miller and Arnison, 1986; Engelhardt et al., 1988). That is why the treatments which release the «masked» mycotoxins can be applied before analysis (Gareis et al., 1990) through the use of the glycosidases. In addition to the use of glycosidases, the procedures of

extraction and purification used before the treatment of glycosidase must be modified to account for the presence of conjugates. For example, the extraction in organic solvents, frequently used in mycotoxins analysis, doesn't allow for recovery of some glycosidic conjugates. The presence of „masked“ mycotoxins was rarely taken into account in the past. In conclusion, a decrease of the mycotoxins concentration in the field doesn't demonstrate the existence of a biological degradation.

### **1.2. Trichotecens detoxification by bacteria capable to produce fermentation**

Lactic acid bacteria (LAB) can be normally encountered in feed and food or are added as pure cultures to different food products. They are considered as not having negative effects, and are involved even in human and animal health improvement (probiotics). LAB have a GRAS status (generally recognized as safe), and it is estimated that 25% from European diet, and 60% from the diet in many developing countries consist of fermented food (Stiles, 1996). The tradition to use LAB in combined food with the recent knowledge about the positive effect on health determined by LAB ingestion suggested that they represent an alternative source to chemical preservatives. Recent studies showed that LAB can be involved also in the diminishing of the mycotoxins concentration.

The idea to decontaminate grains through fermentation was initiated at the beginning of 1980. Ensiling is a traditional technique to preserve feeds by lactic acid fermentation. Fungi from the silage material can produce mycotoxins in aerobic conditions. Anyway, mycotoxins can be also degraded during ensiling. In the last period was realized a great number of studies which investigated the role of lactic acid bacteria (LAB) in reducing the mycotoxins concentration.

Strains of lactic acid bacteria have been used to remove mycotoxins. For example, different strains of lactic acid bacteria (LAB) are capable to bind DON, but the mode of action doesn't seem to be biodegradation, because there wasn't observed the presence of any toxin derivative, and the removal wasn't affected at the non-viable bacteria. The capacity to bind of the selected strains can be used to decrease the toxins bioavailability in the contaminated feeds (Niderkorn et al. 2006).

El-Nezami et al., (2002) investigated the capacity of bacteria *Lactobacillus rhamnosus GG*, *Lb. rhamnosus LC-705* and *Propionibacterium freudenreichii ssp. shermanii JS* to bind seven trichotecens: DON, 3-acetyldeoxynivalenol (3-AcDON), nivalenol (NIV), fusarenone (FX), diacetoxyscirpenol (DAS), T-2 and TH-2. The authors demonstrated that the efficiency of lactic acid bacteria in removal of trichotecens (20 µg/ml) vary according to the bacterial strain and

the toxins; the forms of *Lb. rhamnosus* GG (living or those killed by heat) had an increased efficiency compared to *Lb. rhamnosus* LC-705. The most efficient strain was capable to bind four of the seven tested toxins. The percentage of bound toxins varied between 18% and 93% (El-Nezami et al., 2002). Moreover, HT-2 toxin was bound only by the non-viable cells of *Lb. rhamnosus* GG and LC-705.

Checking the mycotoxin detoxifying potential of 59 probiotic strains, Cheng et al., (2010) found two strains of *Bacillus* which have the capacity to detoxify DON. The detoxification capacity reached the maximum level when the supernatant from the culture of these bacteria was incubated at 37°C, 180 rpm for 12 h, the detoxification rate being 98% and 71.4%, respectively. This rate decreases at the same time with the rising temperature, and the detoxification potential is null at a temperature of 100°C. The authors affirm that the DON detoxification mechanism is bound by some thermo-sensitive substances from the supernatant of these bacilli.

On the other hand, DON wasn't modified during the process of fermentation, and the original quantity was recovered in beer (Scott et al., 1992; Schwarz et al., 1995).

In our laboratory, a feeding trial was conducted to evaluate the effect of a *Lactobacillus* polyculture (LB) on growth and immunological parameters of piglets fed a deoxynivalenol (DON) contaminated diet. A dose of DON (1.8 ppm) was included in a corn-soybean diet provided for ad libitum consumption to twelve weanling piglets for a period of 24 days. The piglets were randomly allotted to one of the following groups: control group (C), *Lactobacillus* group (LB), deoxynivalenol group (DON), deoxynivalenol and *Lactobacillus* group (DON + LB). During the experiment, 50 mL of the *Lactobacillus* polyculture (Suinlact® INCDBNA Balotesti, Romania) was given daily to the pigs from the LB and DON+LB groups. No effect of the treatment was observed concerning body and organ weight of the animals, the serum concentration of glucose, total protein, urea, creatinine and the activity of hepatic enzymes ALT, AST and PA. DON induce in serum a decrease of the cholesterol, LDL, triglycerides and an increase of Ca, K, Na, parameters that were restored after the LB treatment. Comparing with the control, DON induces an increase of: lymphocyte proliferation, liver cytokine synthesis (TNF- $\alpha$  and IL-8), intracellular oxidative activity of the granulocytes and IgM synthesis. DON doesn't affect the proportion of the lymphocytes subsets in the blood of piglets from the DON group. The LB supplement, significantly alleviate the toxic effects of DON concerning the: lymphocyte proliferation, liver cytokine synthesis (TNF- $\alpha$  and IL-8), intracellular oxidative activity of the granulocytes. In conclusion, these results show that the LB polyculture could counteract some toxic effects of DON administration.

### 1.3. Trichotecens biotransformation by symbiotic microflora of animals

#### 1.3.1. Trichotecens biotransformation by bacteria from the ruminal fluid

It is known that in the case of trichotecens the **12, 13- epoxide ring** is responsible for the toxic action of these toxins, because the removal of the epoxy group determines a significant decrease of mycotoxins toxicity.

Ruminants are less susceptible to the action of some mycotoxins compared to monogastric animals. This fact suggests that the process of mycotoxins detoxification takes place in the rumen and was confirmed for a number of mycotoxins with different structures, indicating the presence of different catalytic activities in the ruminal fluid. One example is the study of Fuchs et al., (2002) which investigated the capacity of a microbial feed additive, isolated from the ruminal fluid, BBSH 797 bacterial strain, to degrade the following trichotecens: T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, scirpentriol and diacetoxyscirpenol. The bacterial strain BBSH 797 is a Gram-positive bacteria, anaerobic, with size of 0.2- 0.4 x 1- 1.5  $\mu\text{m}$ , and which is found as independent bacteria or grouped in chains of up to 100  $\mu\text{m}$ . In the case of T-2 toxin treatment with BBSH 797, the toxin was partially hydrolyzed into HT-2 toxin. Treating HT-2 toxin with BBSH 797 determined an almost complete transformation into its de-epoxy form. Scirpentriol is also transformed into a non-toxic metabolite- de-epoxy-scirpentriol. Observing the microbial detoxification mechanisms of type A trichotecens by BBSH 797, the authors stressed the fact that, in most cases, the first step is transformation of an acetyl group into a hydroxyl group through **de-acetylation**, or it is produced the hydrolysis of other ester groups. The second important step for detoxification is represented by the transformation of epoxide into a double bond through **de-epoxidation**.

Also, Swanson et al. (1987) demonstrated that de-epoxidation of DON, diacetoxyscirpenol, and T-2 toxin can be produced in the presence of ruminal microorganisms obtained from a fistulated cow. Some authors described the reaction of de-epoxidation realized by the ruminal or intestinal flora (Yoshizawa et al. 1983, 1994; He et al., 1992; Kollarczik et al., 1994). Microorganisms from the cow ruminal fluid transform DON into 3a, 7a, 15-trihydroxytrichotec-9, 12-diene-8-one (- de-epoxy-DON), named **DOM-1** – Figure 1 (Yoshizawa et al. 1983). DON de-epoxidation by the ruminal fluid was reported also by Cote et al., (1986), and He et al., (1992). It seems that the microorganisms from the ruminal fluid can have functions of de-acetylation and de-epoxidation, transforming 3 acetyldeoxynivalenol (3ADON) into DON and DOM-1 (King et al. 1984). These transformations by the ruminal fluid were

also observed when the animal was fed with a diet containing DON and 3ADON (Binder et al., 1998).

BBSH 797 strain of *Eubacterium* is by far the most intense studied, being capable to transform trichotecens. The strain was isolated from the bovine ruminal fluid, and its primary function was to transform DON into DOM-1 *in vitro* and *in vivo* (Binder et al. 1998; Schatzmayr et al. 2006a; Schatzmayr et al. 2006b). At present there is on the market an additive for animal nutrition based on BBSH 797. The bacterial polycultures coming from the rumen of fistulising cows present both a deacetylation function, and a de-epoxidation function (Swanson et al. 1987a, 1987b).

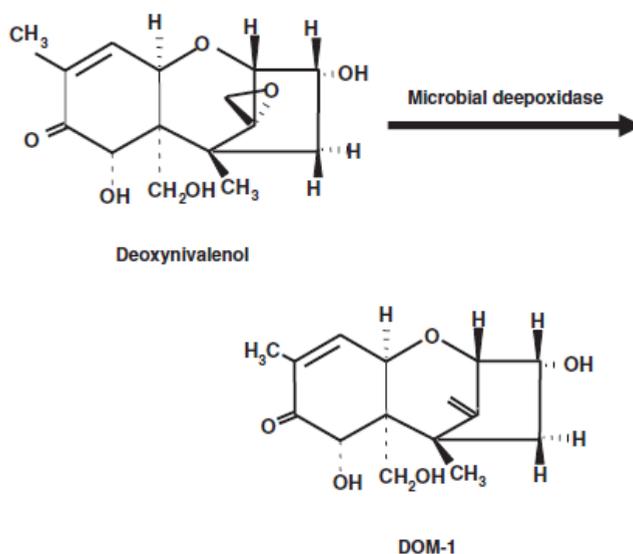


Figure 1. Microbial transformation (*Eubacterium sp.*) of deoxynivalenol to non-toxic metabolites

The transformations produced by microorganisms were obtained after the tested mycotoxins were anaerobically incubated with the bacterial suspensions in a concentration of  $4.9 \times 10^7$  cells/ml at  $38^{\circ}\text{C}$  for 24 or 48 h, DON being transformed into DOM-1.

Beside cows, transformation of DON and other trichotecens by the digestive tract microflora was demonstrated also in the ruminal fluid of sheep (Westlake et al., 1989).

On the other hand, other experiments realized with gastrointestinal microflora coming from sheep and cattle couldn't demonstrate trichotecens de-epoxidation in the ruminal fluid (Kiessling et al., 1984; Munger et al., 1987). Microorganisms from the sheep ruminal fluid presents a de-acetylation function (Kiessling et al., 1984), but DON wasn't transformed by de-

epoxidation. The inability to detect de-epoxidation can be due to the differences between individuals at the level of microflora or to the suboptimal conditions during anaerobic incubation *in vitro*.

### **1.3.2. Trichotecens detoxification by bacteria isolated from poultry digestive tract**

Among microorganisms scanned for their ability to degrade DON, some were found capable to convert DON to much less toxic compounds. A very efficient de-epoxidation activity was obtained when DON was placed in contact with the content of large intestine in chicken; DON was completely transformed into DOM-1 (He et al., 1992). Yu et al., (2010) isolated and identified, by the PCR-DGGE technique, 10 isolates from the chicken intestine, which were capable to transform DON into DOM-1. The majority of isolates were capable to transform DON, and this activity was stable during sub cultivation. Sequencing of genes 16S rRNA showed that the isolates belong to different bacterial groups: *Clostridiales*, *Anaerofilum*, *Collinsella* and *Bacillus*.

Young et al., (2007) studied biodegradation of DON and other trichotecens by the microorganisms from the chicken intestine. Subcultures made from polycultures or isolates of LS100 and SS3 were used for the biodegradation tests, and the studies showed that all the 12 trichotecens were metabolized. De-acetylation and de-epoxidation seem to be the main detoxification metabolic pathways (Young et al., 2007). The microbial treatment of acylated trichotecens led to different results. In the presence of an acetyl group (ex: 3ADON, 15ADON and X fusarenone), de-acetylation was made in favor of de-epoxidation. De-epoxidation was the main reaction for HT-2 toxin and T-2 triol degradation, while T-2 toxin suffered de-acetylation only.

It was demonstrated that the de-epoxidation function of microorganisms from the chickens intestine can vary very much according to the species, individual, and intestinal regions.

### **1.3.3. Trichotecens detoxification by bacteria isolated from the digestive tract of mammals**

As it was previously showed, DON detoxification in the presence of bovine ruminal fluid is done *in vitro* through reductive de-epoxidation, leading to DOM-1 (King et al., 1984). The same metabolite was also found in the rat urine and feces (Yoshizawa et al., 1983; Lake et al., 1987). Anyway, these experiments were performed by feeding animals with DON and it wasn't clear if the transformation results from the metabolic process or the activities of microflora from the digestive tract. The proof that this conversion of DON into a de-epoxy derivative in rats was rather realized by microorganisms than the animal tissue, and was made by elimination of intestinal microflora by

treatment with antibiotics, and by incubating DON with the intestinal content *in vitro* (Worrell et al., 1989).

Beside rats, transformation of DON and other trichotecens by digestive tract microflora was demonstrated also in pigs (Kollarczik et al., 1994). In an experiment made on weaned piglets intoxicated with 500 µg/kg ochratoxin and 200 µg/kg zearalenone, and treated with a combination of minerals, *Eubacterium* (BBSH 797) and *T. mycotoxinivorans* (MTV), Schatzmayr et al., (2005) showed that the treatment with MTV determined an improvement of the zootechnical performances affected by toxin, findings which were confirmed by both the clinical results (reduction of rectal prolapse and diarrhea), and the histopathological results.

It was demonstrated that the de-epoxidation function of microorganisms from the mammals' intestine can vary very much according to the species, individual, and the intestinal regions. These variations were observed in the activity of de-epoxidation of DON by the microorganisms from the pig intestine (He et al., 1992; Kollarczik et al., 1994).

Other experiments made with gastrointestinal microflora couldn't demonstrate trichotecens de-epoxidation in the faeces of horses and dogs (Swanson et al., 1988). The incapacity to detect de-epoxidation can be due to differences between individuals at the level of microflora or suboptimal conditions during anaerobic incubation *in vitro*.

#### **1.3.4. Trichotecens detoxification by the symbiotic microflora of invertebrates**

The insects, nematodes and other invertebrates which feed with the cultures infected with fungi (even with hyphae and spores) in the field or during storage are exposed to high concentrations of mycotoxins. These invertebrates are resistant to the toxins produced by the fungal species they consume. For example, *Carabus* which feed themselves with corn are resistant to the majority of mycotoxins produced by *Aspergillus spp.* and *Fusarium spp.*, which contaminate corn (Dowd, 1995), and some species of *Collembola* don't present toxic symptoms after they were fed with high concentrations of DON, T-2 toxin and zearalenone (Karlovsy, 1999).

Insect's response to the fungal toxins can vary quite a lot (Dowd et al., 1989). Microbial symbionts can be responsible for the mycotoxins detoxification at the resistant species. Actually, the symbionts can be considered promising sources of enzymes with a role in detoxification (Dowd, 1992). 4-monoacetoxyscirpenol was hydrolyzed to scirpentriol by the digestive system of *Carpophilus hemipterus* larva which eats fungi (Dowd and Van Middlesworth, 1989). The transformation was considered by the authors as being a detoxification process, and a metabolic adaptation for feeding with

fungi, although later Grove and Hosken (1975) observed that scirpentriol is 30 times more toxic for human cell lines compared to monoacetoxyscirpenol. Anyway, monoacetoxyscirpenol used by these authors was acetylated at position 15, while that one used by Dowd and Van Middlesworth (1989) was acetylated at position 4. The role of de-acetylation of 4-monoacetoxyscirpenol in the metabolic adaptation of *Carpophilus hemipterus* to the fungal diet remains unclear.

#### 1.4 Trichotecens detoxification by environmental bacteria

The effects of bacterial populations isolated from the environment on T-2 toxin and other trichotecens have been described in literature. Thus, Beeton and Bull (1989) investigated the role of bacterial polycultures and monocultures, as agents for detoxification and biodegradation of T-2 toxin and related trichotecens.

Bacterial populations capable of detoxification and biodegradation were collected from different niches, such as soils and litter, rivers, waste waters. Experiments showed that 85% from the isolates coming from soils and water were capable to use T-2 toxin as the only carbon and energy source, and this activity was associated with removal of T-2 toxin toxicity. Two populations, TS4 and KS10, degraded the nucleus of trichotecens within 24 to 48 h (Beeton and Bull, 1989). In the majority of isolates, the main path to degradation of T-2 toxin involved the lateral cleavage of acetyl group in order to produce HT-2 toxin and T-2 triol. It was also identified a partial degradation of T-2 toxin which involves conversion to neosolaniol and later to 4-deacetyl neosolaniol. The co-metabolic interaction between species is suggested as being a significant factor in T-2 toxin degradation. Ueno et al., (1983) used strain 114-2 of *Curtobacterium sp.* as bacteria which assimilates T-2 toxin. During incubation with *Curtobacterium sp. strain 114-2* in a minimal medium T-2 toxin was converted to T-2 triol through HT-2 toxin, and T-2 triol was later assimilated by bacteria without the formation of neosolaniol and T-2 tetraol. This study indicated that the strain of *Curtobacterium sp.* and other bacteria isolated from soil were capable to degrade trichotecens (Ueno et al., 1983). Incubation of T-2 toxin with a strain of *Bacillus megaterium* demonstrated that this bacterium is capable to bind 8% from the quantity of toxin (Engler et al., 2000).

DON is the most common mycotoxin which contaminates the grains and is chemically stable, but doesn't accumulate in soil, which suggests the possibility of DON degradation in the nature (Völkl et al., 2004). Among the 1285 microbial cultures obtained from soils coming from farms, seeds and grains, insects and other sources, only a mixed culture was capable of transforming DON into 3-keto-DON (Völkl et al. 2004). The culture was also capable to

transform 3ADON, 15ADON and FUS. Anyway, microorganisms responsible for biotransformation were not identified. Moreover, microorganisms from soil are capable to transform DON and 3ADON into five unidentified metabolites (Binder et al., 1998).

The strain *E3-39 Agrobacterium–Rhizobium* was isolated from samples of soil through a method of enrichment which uses DON as the only source of carbon in a medium which contains mineral salts (Shima et al. 1997). The strain transforms DON into 3-keto-DON in anaerobic conditions. The transformation activity of the strain is stable in time, and the enzyme responsible of the microbial transformation was found in culture and in the cell filtrate, but not in the cell extract (Shima et al., 1997). Six soils coming from the agricultural lands capable to transform DON into certain metabolic products were obtained after enrichment using corn contaminated with *F. graminearum*. These enriched soils can transform DON from the culture media after incubation at 28°C for 3 days (Zhou 2008). A bacterial strain isolated from the soil samples presented a strong capacity to transform DON. In aerobic conditions, the bacterial strain transforms DON into at least two products: the major product is a stereo-isomer of DON, and the minor one is 3-keto-DON.

## **2. Fungal detoxification of trichotecens**

### **2.1 Trichotecens detoxification by yeasts capable to produce fermentation**

Fermentation is one of the oldest forms of processing and storage of food in the world, as early as 7000 years ago in Babylon (Battcock and Azam-Ali, 1998). Moreover, fermentation is one of the easiest and cheapest food storage methods, beside its benefits on the nutritional and organoleptic qualities of fermented foods. Consumption of fermented foods is deeply rooted in African and Asian culture, where fermented grains represent an important part of the daily diet. Fermentation is made by natural microbiota of raw materials, microorganisms which come from the fermentation equipments or by the cultures added from outside. The predominant microorganisms which produce grains fermentation are, beside lactic acid bacteria, the yeasts, especially *Saccharomyces cerevisiae* and *Candida krusei* (Jespersen et al., 2003). It was demonstrated that some strains of *S. cerevisiae* var. *boulardii* have a probiotic activity (Coeuret et al., 2004). Recently, it was showed that a strain of *S. cerevisiae* isolated from African indigenous food products has a promising probiotic potential (van der Aa Kuhle et al., 2004). Moreover, the yeasts were administered to the animals since hundreds of years, and the commercial products based on yeasts are produced on a commercial scale for animal nutrition (Celyk et al., 2003). Yeasts have a huge potential to be used in decreasing mycotoxin effects in foods based on grains and in feeds for animals.

In a FAO/WHO workshop realized in the fermentation field was stressed the necessity of obtaining scientific data on different aspects of foods fermentation, including binding of toxins (Nout and Motarjemi, 1997).

Mannan-oligosaccharides derived from the cells of *S. cerevisiae* demonstrated a little capacity of binding deoxynivalenol (Shetty and Jespersen, 2006). It was later showed that esterified glucomannane offers protection to chickens exposed to ochratoxin and T-2 toxin, beside the protection provided against aflatoxin (Raju and Devegowda, 2000), and reduces the toxic effects of fusariotoxins in horses (Raymond et al., 2003). Supplementing the feed with glucomannane (GM, extracted from the yeast cell wall, Alltech Inc.) prevents some alterations induced by DON in piglets on the neuronal transmission and serum concentrations of immunoglobulin (Swamy et al., 2002). In turkeys, the same polymer counteracts the effects induced by a feed contaminated with DON (3.9 µg/g), ZEN (0.67–0.75 µg/g), 15-AcDON (0.34 µg/g) and HT-2 (0.078–0.085 µg/g) at the level of intestinal immunity, cell proliferation and lymphoid organs (Girish et al., 2010). The potential effects of ethanolic fermentation made by yeasts on fusariotoxins were studied by Flesch and Voight-Scheurman (1994), who studied the effect of alcoholic fermentation of must on trichotecens concentration. There were identified derivatives which didn't contain the epoxide group. It was also suggested the fact that yeast probably produces ligases and keto-enol tautomerase. Later it was established the fact that a significant quantity of mycotoxins (approximately 20%) was taken over by yeast. Because the ring 12, 13-epoxide of these compounds is responsible for their toxicity, a de-epoxidation produced by specific enzymes (de-epoxidases) is responsible for the significant loss of toxicity.

## **2.2. Trichotecens decontamination by yeasts isolated from invertebrates**

A symbiotic yeast strain, *Pseudotaphrina kochii*, capable to degrade a diversity of mycotoxins and xenobiotics was isolated from the cigarette beetle. The strain grew on a medium with citrinine, DON, mycophenolic acid, A ochratoxin and sterigmatocystine, as unique source of carbon, and the growth on a medium with zearalenone was described as „variable” (Dowd and Shen, 1990; Shen and Dowd, 1991). On the other hand, Karlovsky et al., (1999) in experiments in which they used the original strain of *P. kochii* (NRRL-18546) couldn't obtain yeast cultures on a minimal solid medium which contained DON or zearalenone, not even after 17 weeks in culture, and they didn't detect a degradation activity of DON or zearalenone- in the cultures made in liquid medium. Possible explanations of this discrepancy are represented by the degeneration of metabolic abilities of the strain in the stock cultures or the

presence of impurities in the agar used by Shen and Dowd, which facilitated the use of DON and zearalenone in these experiments.

### 3. Conclusions and perspectives

The research realized with bacteria and yeast showed a possible difference between the sensibility and selectivity of these microorganisms to mycotoxins (Madhyastha et al., 1994). For example, *Bacillus brevis* was sensitive to eight mycotoxins including zearalenone and ochratoxin A, but it wasn't affected by high concentrations of trichotecens. The yeast *Kluyveromyces marxianus* was sensitive to all trichotecens, but not to other mycotoxins.

The study of mechanisms involved in resistance to mycotoxins (e. g. selective passage through membranes, decomposition by selective enzymes, blocking by formation of complexes, etc.) can be useful in the selection of microorganisms. The control of degradation products and effects of detoxification on nutritive and organoleptic properties are, in each case, a decisive part of this research and its applications. For the efficient use of microorganisms as part of food additives in decontamination certain characteristics are necessary, and this must be done in the presence of different oxygen concentrations and in a complex medium with high level of stability in the gastrointestinal tract at different levels of pH.

Anaerobic microorganisms isolated from the digestive tract are generally suitable for including in food additives, which will act in the intestine of target animals. Survival and adaptation of microorganisms in the digestive tract are the essential factors for a successful detoxification.

In the future the isolation and characterization of more and more microorganisms with degradation or binding properties might lead to the discovery of bacteria and yeasts more efficient in decontamination.

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