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Research on the use of the biotechnology techniques of milk decontamination

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Abstract

Mycotoxin contamination of milk problem is the problem of current interest and has received great attention in the last three decades. It is known that the occurrence of aflatoxin M1 (AFM1) in milk is a public health problem, it has been recently classified by the International Agency for Research on Cancer as a possible human carcinogen factor. Because of this goal, the aim of this paper is to study some lactic cultures in order to reduce the content of AFM1 in milk contaminated. Results indicate significant differences ($p < 0.001$) during the degradation AFM1 thermostat with mesophilic lactic cultures. Thus the potential of certain lactic cultures is promising and open new horizons of research in biotechnology decontamination AFM1.

Keywords: biotechnology , decontamination , aflatoxin M1

1. Introduction

Mycotoxins are naturally produced by filamentous fungi contaminants as by-products of metabolism [1]. Over 100 species of fungi have been shown to produce mycotoxins would naturally associated with the occurrence of various diseases to animals and humans [2,3] ,however the most common mycotoxins occurring in feed which often belong to species of fungi such as *Aspergillus Aries, Fusarium and Penicillium* [4,5]. The occurrence of mycotoxins in food and animal feed depend on many biological factors such as region, season, humidity and temperature [6].

Recently mycotoxins from *Fusarium* species have been identified in conventional manner in grain from temperate zones, since these fungi require a relatively lower temperature than the mycotoxins growing species aflatoxicogenic [7].

However the most important mycotoxins are aflatoxins, ochratoxin, zearalenone and fumonisins and aflatoxins are the most toxic and is currently of particular concern to human health [8,9]. These are natural compounds produced by several species of *Aspergillus* particularly *Aspergillus flavus* and *Aspergillus parasiticus*, are among the most toxic substances known carcinogens that after entering the body are metabolized by the liver to reactive intermediates such as Aflatoxin M1 or M2, often they can be found in dairy products obtained from animals which have ingested the fodder contaminated with aflatoxin B1 or B2 [10].

Currently the occurrence of aflatoxin M1 in milk is a public health problem, it has been classified by the International Agency for Research on Cancer as a possible human carcinogen factor [11]. Exposure to aflatoxin M1 through contaminated milk products is serious public health problem.

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Considering this, some countries have established regulatory restrictions for aflatoxin M1 in particular in milk and milk products sector, these restrictions are different from country to country [12].Considering the toxicity of aflatoxin in milk, the European Commission adopted Regulation EC No. 466/2001 European level of aflatoxin M1 0.050 μg Kg^{-1} [13].

Since aflatoxin contamination is a potential hazard, developing effective methods of detoxification presents a common concern in the scientific community [14], therefore some mycotoxin decontamination methods have been described in the literature, these have included the physical and chemical treatments [15-18]. However there is currently available methods to be effective and practical [19].

Currently is known as food fermentation using lactic acid bacteria was used as a method of preservation for centuries, however data exist in the literature regarding the fact that lactic acid bacteria can reduce fungal growth [20,21]. In addition them were considered that would inhibit the biosynthesis of aflatoxins due to lactic acid or lactic acid metabolites [22]. Several bacterial strains, food or human origin were tested for their ability to bind other mycotoxins [23-25].

Among the most effective methods of detoxification include the biological detoxification wich offering an attractive alternative for the elimination of toxins in safely condition. These methods are mainly based on two major processes, absorption and enzymatic degradation, both can be achieved by biological systems [26,27].

Live microorganisms can absorb or mycotoxin attaching to cell wall components or active internalization and accumulation. Microorganisms deadly can absorb mycotoxins more than viable cells and this can be exploited by creating biological filters decontamination liquids using probiotics to bind and eliminate mycotoxins in the gut.

The enzymatic degradation can be carried out by the enzyme degradation can however be considered to be complete only when the final product is converted into CO2 and water. However enzymatic modification can change, reduce or completely eliminate the toxicity [28].

The aim of this study is to evaluate the potential of the decontamination of the lactic cultures to reducing the content of AFM1 contaminated milk.

2.Materials and methods

2.1.Preparation of samples

Cow's milk was bought from trade with a technical specification wich includes the following physico-chemical and microbiological parameters.

Table 1. Physico-chemical and microbiological characteristics of cow's milk

Acidity (° T) $\bar{x}$ ± S_r*	**Fat (%)** $\bar{x}$ ± Sr*	**Density (g/cm^3)** $\bar{x}$ ± Sr*	**SNF (%)** $\bar{x}$ ± Sr*	**Protein (%)** $\bar{x}$ ± Sr*	**Colony-forming unit UFC Value (·1000 cells· mL^{-1})** $\bar{x}$± Sr*	**Somatic cell count (SCC) (1 000 cells $mL^{-1)}$** $\bar{x}$ ± Sr*
17.23 ± 0.27	3.78 ± 0.01	1.0273 ± 0.01	8.26 ± 0.09	3.10 ± 0.06	35±0.1	3±0.1

* Standard deviation, calculated from results generated under repeatability conditions (pentru trei determinări)

2.2. Lactic cultures

Cultures are coming from CHR. Hansen, and were frozen at -80 °C, the products have the inscription on the label 50U, 200U and 500 microns, and the "U" represents the number of colony forming unit at 37 °C (1 g corresponds to 1 unit "U" representing 5 x 10^{10} colony forming unit g^{-1}).

The cultures used were coded according to the composition (according to technical specification received from milk processor) Table 2.

2.3. Preparation of AFM1 standards

AFM1 was purchased from Charm Sciences Inc. as a solid (0.100 mg vials mL^{-1} AFM1). The stock solutions were diluted in acetonitrile: benzene (98:2).

The amounts of methanol were prepared by evaporation of the acetonitrile: benzene and reconstituted in methanol, AFM1 concentrations were determined spectrophotometrically at 363 nm and stored at 20 ° C.

Table 2. The coding cultures and microbiological composition

Composition	Code culture
Lactococcus lactis subsp. *cremoris* *Lactococcus lactis* subsp. *lactis* *Leuconostoc mesenteroides* subsp. *cremoris* *Lactococcus lactis* subsp. *diacetylactis*.	C1
Streptococcus thermophilus	C2
Lactococcus lactis subsp. *Lactis* *Lactococcus lactis* subsp. *Cremoris*	C3
Streptococcus thermophilu *Lactobacillus delbrueckii subsp. lactis* *Lactobacillus delbrueckii subsp. Bulgaricus*	C4

2.4. Quantitative determination of AFM1

Aflatoxin analysis was performed according with ISO 14501: 2007, samples were injected into HPLC analytical column with a reverse phase (220 x 4.6 mm, 5 mm), retention times were about 6 minutes to AFM1.

In the chromatogram of the peaks of aflatoxin were highlighted in a fluorescence detector at 365 nm (excitation) and 435 nm (emission), and a UV detector at 360 nm and quantified by software. The test was made at 40 ^{0} C with an injection volume of 10 mL.

2.5. Experimental data

Evaluation of the degree of binding between AFM1 in cow milk ,artificially contaminated at a concentration of 0.1 mg L^{-1} AFM1. and commercial crops has been achieved on various levels of inoculum (2.5 $\cdot 10^{10}$, 5 $\cdot 10^{10}$ and 10^{10} CFU of 7.5 mL^{-1}).

3. Results and discussion

In Table 3 is shown the variation of the degree of binding the AFM1 during the 6 hours of incubation at 37 °C in the different cultures and inoculation levels, for artificially contaminated milk with AFM1 at a concentration of 0.100 mg mL^{-1}.

Table 3. Variation of the level of AFM1

Used culture	**Amount of culture used (no. of units) / no. strains inoculated into 1000 mL of milk at 37 ^{0} C min. (CFU mL $^{-1}$)**	**Degree of binding of AFM1 by lactic cultures (%) ± *RSD***						
		Time (h)						
		0	**1**	**2**	**3**	**4**	**5**	**6**
C1	2,5x 10^{10}	0	14,4 ± ,3	24,9 ± 0,4	32,3 ± 0,4	33,2 ± 0,3	41,3 ± 0,2	51,1± 0,3
	5 x 10^{10}	0	18,7 ±0,2	27,9 ± 0,3	33,7 ± 0,2	36,9 ± 0,2	43,8 ± 0,1	54,8 ± 0,2
	7,5 x 10^{10}	0	18,9 ±0,2	28,8 ± 0,4	34,8 ± 0,3	48,5 ± 0,4	54,9 ± 0,1	59,9 ± 0,2
C2	2,5x 10^{10}	0	8,7 ± 0,2	18,5 ± 0,5	24,2 ± 0,3	26,9 ± 0,2	33,9 ± 0,3	35,9 ± 0,3
	5 x 10^{10}	0	11,7 ± 0,4	20,9 ± 0,1	29,7 ± 0,2	32,9 ± 0,2	33,8 ± 0,2	39,8 ± 0,1
	7,5 x 10^{10}	0	14,9 ± 0,5	32,7 ± 0,2	39,5 ± 0,1	44,5 ± 0,3	49,9 ± 0,2	53,9 ± 0,1
C3	2,5x 10^{10}	0	7,7 ± 0,2	18,5 ± 0,2	22,2 ± 0,1	26,9 ± 0,2	32,5 ± 0,1	34,2 ± 0,1
	5 x 10^{10}	0	9,7 ± 0,3	19,5 ± 0,2	26,7 ± 0,2	30 ± 0,2	37,3 ± 0,2	39,8 ± 0,1
	7,5 x 10^{10}	0	12,9 ± 0,3	24,7 ± 0,2	29,5 ± 0,1	34,5 ± 0,3	39,9 ± 0,1	42,1 ± 0,1
C4	2,5x 10^{10}	0	13,2 ± 0,1	25,8 ± 0,3	30,1 ± 0,1	32,5 ± 0,2	40,5 ± 0,1	41,1 ± 0,2
	5 x 10^{10}	0	16,5 ± 0,2	26,5 ± 0,1	32,5 ± 0,3	34,2 ± 0,3	42,2 ± 0,4	42,3 ± 0,5
	7,5 x 10^{10}	0	18,5 ± 0,2	26,7 ± 0,3	33,9 ± 0,2	38,2 ± 0,1	44,5 ± 0,4	48,5 ± 0,1

RSD* Relative standard deviation for three determinations

Data demonstrate that incubation time have significantly influence (P <0.001) to AFM1 concentration compared to other parameters (type and degree of inoculation culture).

The lowest concentration of AFM1 after incubation for 6 hours at 37 ^{0}C was when cultures inoculated C1 to a degree of 7.5 x 10^{10} and the lowest in the case of C3 culture.

From the point of view of the composition of the lactic acid strains, only *Lactococcus lactis* subsp. lactis was present in both lactic cultures, although C1 culture is a culture with mesophilic flora in *Lactococcus lactis*, in C3 strain culture prevails subsp. cremoris, this was probably responsible for binding strain AFM1 in a higher percentage than other cultures.

Similar studies were done by El-Nezami, Kankaanpaa [29] wich have found that gram positive bacteria (five strains of *Lactobacillus* and *Propionibacterium*),were more effective than *E. coli* in the removal of AFB1 in a liquid medium.

However observations made by [30] maintain that specific bacteria in yogurt can provide decontamination of AFM1 in milk. This study is based on assessing the degree of binding of AFM1 with *Lactobacillus delbrueckii* subsp and bulgaricus and Streptococcus thermophilus CH-2 ST-36 in phosphate-buffered saline (PBS).An explanation in terms of specificity bacterial strains constitute a bacterial concentration, which influences the elimination AFB1. Thus, different trough levels have been reported to be able to eliminate only 13% of AFB1 in an hour [31].

A possible explanation of the nature of AFB1 binding of the sites on the surface of lactobacilli is the membrane wall peptidoglycan cell and polysaccharide [32,33].This was confirmed later by SJ, Haskard [34] who studied different cell wall components (exopolysaccharides and peptidoglycan) of bacteria and concluded that peptidoglycan represented binding sites to AFB1 and heat treatments were denatured proteins and influenced the formation of Maillard products.

Acid treatments were broken glycosidic linkages from polysaccharides and amino bonds of peptides and proteins and they have increased the size of pores in the peptidoglycan stratum of bacterial surface [33]. This process could allow binding of aflatoxin B1 in the cell wall and could explain the increased binding capacity of bacteria destroyed by acid treatment compared with viable bacteria.

4. Conclusion

Aflatoxins are a serious threat to animal and human health and efforts continue to be devoted worldwide to prevent or eliminate them. The first step to preventing the formation of AFs is to control or prevent the growth of fungus.

To prevent and effectively control aflatoxicosis recent research has succeeded to demonstrate efficiency to adsorbents treatments to reduce AFB1 significantly in animal feed.

According to the results of experiments until now, the micro-organisms are the main living organisms that apply for biodegradation of mycotoxins. Microorganisms such as bacteria from soil or water, or enzymes isolated from microbial systems can degrade group members aflatoxin with varied efficiency. Some aflatoxin producing fungi *Aspergillus* species have the ability to degrade own mycotoxins synthesized. However some yeasts and lactic acid bacteria are biological sinks prevent the transfer of aflatoxins in the intestinal tract of humans and animals.

Field detoxification of aflatoxins by microorganisms seems promising. Therefore this approach should give more attention to these organisms and enzymes produced by them.

It is also very important to evaluate the potential of certain strains of lactic probiotics and how yeasts linking aflatoxins and the factors affecting the stability of the complex formed toxin.

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References

1. Beltrán, E., et al., Development of sensitive and rapid analytical methodology for food analysis of 18 mycotoxins included in a total diet study. Analytica Chimica Acta, (0).
2. Rechcigl, M., *CRC handbook of naturally occurring food toxicants*. 1983: CRC Press, Inc.
3. Sharma, R.P. and D.K. Salunkhe, *Mycotoxins and phytoalexins*. 1991: CRC Press, Inc.

4. Kumar, V., M. Basu, and T. Rajendran, Mycotoxin research and mycoflora in some commercially important agricultural commodities. *Crop Protection*, **2008**, *27*(6), 891-905.
5. Bryden, W.L., Mycotoxins and mycotoxicoses: significance, occurrence and mitigation in the food chain. General, *Applied and Systems Toxicology*, **2009**.
6. Dashti, B., et al., Levels of aflatoxin M1 in milk, cheese consumed in Kuwait and occurrence of total aflatoxin in local and imported animal feed. *Food Control*, **2009**. *20*(7), 686-690.
7. Placinta, C.M., J.P.F. D'Mello, and A.M.C. Macdonald, A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. *Animal Feed Science and Technology*, **1999**, *78*(1–2), 21-37.
8. Schappert, K. and G. Khachatourians, A yeast bioassay for T-2 toxin. *Journal of microbiological methods*, **1984**, *3*(1), 43-46.
9. Williams, J.H., et al., Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *The American journal of clinical nutrition*, **2004**, *80*(5), 1106-1122.
10. Unusan, N., Occurrence of aflatoxin M1 in UHT milk in Turkey, *Food and Chemical Toxicology*, **2006**, *44*(11), 1897-1900.
11. Boudra, H., et al., Aflatoxin M< sub> 1</sub> and Ochratoxin A in Raw Bulk Milk from French Dairy Herds. *Journal of dairy science*, **2007**, *90*(7), 3197-3201.
12. Ruangwises, N. and S. Ruangwises, Aflatoxin M1 contamination in raw milk within the central region of Thailand. *Bulletin of environmental contamination and toxicology*, **2010**, *85*(2), 195-198.
13. European Commission, R.E., No. 466/2001 of 8 March 2001, O.J.E.U.L. 1–13., Editor. 2001.
14. Akbas, M.Y. and M. Ozdemir, Effect of different ozone treatments on aflatoxin degradation and physicochemical properties of pistachios. *Journal of the Science of Food and Agriculture*, **2006**. *86*(13), 2099-2104.
15. Herzallah, S., K. Alshawabkeh, and A.A. Fataftah, Aflatoxin Decontamination of Artificially Contaminated Feeds by Sunlight, γ-Radiation, and Microwave Heating. *The Journal of Applied Poultry Research*, **2008**, *17*(4), 515-521.
16. Coker, R.D., 12 The chemical detoxification of aflatoxin-contaminated animal feed. *Natural Toxicants in Food*, **1998**, *2*, 284.
17. Piva, G., et al., Detoxification methods of aflatoxins. *Zootecnica International*, **2000**, *23*(7/8)
18. Ketney, O., T. Ovidiu, and T. Mihaela. *Variation of aflatoxin M1 in milk at different doses of ultraviolet radiation*. in *Integrated systems for agri-food production (SIPA 11)*. 2010. ISBN 978-615-5097-26-3.
19. Peltonen, K.D., et al., Binding of aflatoxin B1 by probiotic bacteria. *Journal of the Science of Food and Agriculture*, **2000**, *80*(13), 1942-1945.
20. Mokoena, M., P. Chelule, and N. Gqaleni, The toxicity and decreased concentration of aflatoxin B1 in natural lactic acid fermented maize meal. *Journal of applied microbiology*, **2006**, *100*(4), 773-777.
21. Ketney, O., et al., Reserches regarding aflatoxin production in Feta chesse by direct contamination with Aspergillus flavus. *Journal of agroalimentary processes and technologies*, **2008**, *2*, 442-445.
22. Gourama, H. and L.B. Bullerman, *Aspergillus flavus* and *Aspergillus parasiticus*: Aflatoxigenic fungi of concern in foods and feeds: A review. *Journal of Food Protection®*, **1995**, *58*(12), 1395-1404.
23. El-Nezami, H., et al., Removal of common *Fusarium toxins* in vitro by strains of *Lactobacillus* and Propionibacterium. *Food Additives & Contaminants*, **2002**, *19*(7), 680-686.
24. El-Nezami, H., et al., Binding Rather Than Metabolism May Explain the Interaction of Two Food-Grade Lactobacillus Strains with Zearalenone and Its Derivative ά-Zearalenol. *Applied and Environmental Microbiology*, **2002**, *68*(7), 3545-3549.
25. Styriak, I. and E. Conková, Microbial binding and biodegradation of mycotoxins. *Veterinary and human toxicology*, **2002**, *44*(6), 358-361.
26. Halasz, A., et al., Decontamination of Mycotoxin-Containing Food and Feed by Biodegradation. *Food Reviews International*, **2009**, *25*(4), 284-298.
27. Yin, Y.-n., et al., Biological control of aflatoxin contamination of crops. *Journal of Zhejiang University SCIENCE* B, **2008**, *9*(10), 787-792.
28. Magan, N. and M. Olsen, *Mycotoxins in Food: Detection and Control*. 2004: Woodhead Pub.
29. El-Nezami, H., et al., Physicochemical alterations enhance the ability of dairy strains of lactic acid bacteria to remove aflatoxin from contaminated media. *Journal of Food Protection®*, **1998**, *61*(4), 466-468.
30. Belgin Sarimehmetoğlu and Özlem Küplülü, Binding ability of aflatoxin M1 to yoghurt bacteria. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, **2004**, *51*, 195-198.

31. Bolognani, F., C. Rumney, and I. Rowland, Influence of carcinogen binding by lactic acid-producing bacteria on tissue distribution and in vivo mutagenicity of dietary carcinogens. *Food and chemical toxicology*, **1997**, *35*(6), 535-545.
32. Haskard, C., C. Binnion, and J. Ahokas, Factors affecting the sequestration of aflatoxin by *Lactobacillus* rhamnosus strain GG. *Chemico-biological interactions*, **2000,** *128*(1), 39-49.
33. Haskard, C.A., et al., Surface binding of aflatoxin B1 by lactic acid bacteria. *Applied and Environmental Microbiology*, **2001**, *67*(7), 3086-3091.
34. SJ, L., et al., Binding of aflatoxin B1 to cell wall components of Lactobacillus rhamnosus strain GG. Food additives and contaminants, **2004**, *21*(2), 158-164.