Pol. J. Environ. Stud. Vol. 31, No. 5 (2022), 4985-4988
DOI: *10.15244/pjoes/150048* ONLINE PUBLICATION DATE: 2022-08-08

Short Communication

Biocontrol of Aflatoxin through Biodegradation by Using Environment Friendly Microbes

Ali Akbar[1]*, Ghulam Ishaq Khan[1], Muhammad Shafee[2], Abdul Samad[2], Hamid Masood[3], Shabir Ahmad Khan[2]

[1]Department of Microbiology, University of Balochistan, Quetta, Pakistan
[2]Centre for Advanced Studies in Vaccinology & Biotechnology (CASVAB) University of Balochistan, Quetta, Pakistan
[3]Department of Microbiology, Quaid-I-Azam University Islamabad, Pakistan

Received: 28 March 2022
Accepted: 14 May 2022

Abstract

Aflatoxins are secondary metabolites of fungal species usually produced in tough environmental conditions. The interruption of fatty acid formation results in aflatoxins production. It is equally important in food and feed due to its health complication for animals and human beings. Biodegradation through environment friendly microbes is an acceptable strategy for the control of mycotoxins. A study has been designed to evaluate the environment friendly bacterial species for the biodegradation of aflatoxins. Specific double strength broth media (BHI, TSB, MRS and SDB) were mixed with standard Aflatoxins B1 to obtained 30 ppb final concertation. Different microbial species (*Rhodococcus erythropolis*, *Bacillus licheniformis*, *Lactobacillus Pentosus*, *Lactobacillus casei*, *Aspergillus niger* and *Saccharomyces crevice*) were added to the mixture containing aflatoxins and incubated for 48-72 h. The concentration of the aflatoxins in the mixture was confirmed with Agra strip, Agra quant and HPLC at initial and final time of incubation.

Results showed that *Rhodococcus erythropolis* and *Bacillus licheniformis* can degrade or alter the structure of aflatoxins to the undetectable level of the Agra strip (cutoff value less than 4 ppb) and Agra quant (cutoff value less than 2 ppb), these results were double confirmed with the of HPLC, which showed the reduction of aflatoxins to 0.57 and 0.95 ppb by *Rhodococcus erythropolis* and *Bacillus licheniformis* respectively. These results conforming the biodegradation of aflatoxins B1 used in the study. The organisms used in the study are environment friendly and can have the potential to decrease aflatoxins to the acceptable consumption level. Uses of these microbes for biodegradation will have positive impacts over the biocontrol strategies for mycotoxin.

Keywords: decontamination of mycotoxin, animal feed contamination, mycotoxigenic fungi, food safety

*e-mail: aliakbar.uob@gmail.com

Introduction

Mycotoxins are secondary metabolites of fungal species, produce by some toxigenic strain of fungi such as fusarium, penicillium and aspergillus [1]. The mycotoxin contamination in food and feed exert significant agriculture loses and contribute to animal and human health problem worldwide [2]. Aflatoxins are produce by aspergillus species such as *Aspergillus parasiticus*, *Aspergillus nomius* and *Aspergillus flavus* [3]. The toxicity of aflatoxin is high in both acute and chronic cases in human and animals. It cause mutation in DNA, nephritis, inflammation in liver, immune suppression and other carcinogenic effects [4]. Along with other control strategies applied for the control of aflatoxins, the biodegradation of aflatoxin can be the good choice to control or reduce it to the acceptable level in food and feed [5, 6]. Different group of microorganism such as bacteria and fungi can degrade aflatoxin through their enzymatic activity [7]. Fermentative bacteria are appropriate candidates for the biological detoxification of different mycotoxins by exploiting there potential of enzymatic degradation of different bonds in the mycotoxins molecules [8].

The mechanism of action of probiotic bacteria on mycotoxigenic fungi is based on the competition for physical space and nutrients intake which are required for the growth of probiotics bacteria [9, 10]. The required nutrients for these microorganisms obtained via lysis the living or dead cells or other microorganisms, which were available in the environment [11]. In this study we determined the biodegradation potential of different bacterial and fungal species against the aflatoxin.

Materials and Methods

Aflatoxin Biodegradation

Different microbial cultures namely *Rhodococcus erythropolis*, *Bacillus licheniformis*, *Lactobacillus pentosus, Lactobacillus casei*, *Aspergillus niger* and *Saccharomyces cerevisiae* from existing culture bank of our laboratory were used which were previously isolated form different sources and preserved. All the bacterial species were analyzed for its ability to degrade the aflatoxins.

Aflatoxins B1 of 30 ppb was prepared by introducing the toxins in the double strength specific broth media for each organism and microbes as a biomass roughly equal to 1×10^4 - 1×10^5 CFU/mL were introduced and incubated for 48-72 h. The concentration of aflatoxins was checked with the help of Agra strip (Romer Labs. USA) and Agra quant (Romer Labs. USA) with a cutoff value of 4 and 2 ppb respectively at initial and final time of incubation. The microbes that reduced the value of aflatoxins to undetectable limits of aflatoxins by Agra strip and Agra Quant test were analyzed further with the help of HPLC for detecting the final concentration of biodegraded aflatoxins following the procedure adopted by [12]. The media with aflatoxins and no bacterial species, and bacterial species inoculated to media with no aflatoxins were used as a control.

Statistical Analysis

All the experiments were done in triplicate and SPSS 22 were used for statistical analysis. For the comparison between the aflatoxin stranded and tested samples, data were analyzed through one way ANOVA with 5% of level of significance.

Results and Discussion

The aflatoxins biodegradation experiments were conducted using *Rhodococcus erythropolis*, *Bacillus licheniformis*, *Lactobacillus Pentosus*, *Lactobacillus cassia*, and *Saccharomyces cerevisiae*. All these microorganisms were interacted with mycotoxin pure standard in broth medium and incubated for 36 to 72 hours at 37ºC. The initial conformation was done through the Agra strip (cutoff value less than 4 ppb) and Agra quant (cutoff value less than 2 ppb). The results showed that the *Rhodococcus erythropolis* and *Bacillus licheniformis*, are able to degrade aflatoxins within 36 to 72 hours of their interaction and incubation. The rest of microorganism such as (*Lactobacillus Pentosus*, *Lactobacillus Casei, Aspergillus niger,* and *Saccharomyces cerevisiae*) were negative for the degradation of Aflatoxin. The tests were compared with the positive and negative control for aflatoxins presence and microbial growth. The biodegradation of Aflatoxin through *Rhodococcus erythropolis* and *Bacillus licheniformis* were confirmed with the help of HPLC by subjecting the testing mixture to HPLC analysis. It was found that 30 ppb of aflatoxin which were initially added to the test mixture of media and microbes were reduced by *Rhodococcus erythropolis* to 0.57 ppb and 0.95 by *Bacillus licheniformis*. The peak areas of test samples chromatographs (Fig. 1a, b) were compared with the HPLC chromatograph of standard aflatoxins used in the study. These results of HPLC conforming the biodegradation of aflatoxins B1 used in the study. Similar studies was conducted by Cserháti et al. [13] and Krifaton et al. [14] reported the successful biodegradation of aflatoxins B1 using three different Rhodococcus species in their studies. Different species of this genus has been used for the biodegradation of different mycotoxins particularly aflatoxins B1 [15]. In another study Campos-Avelar et al. [16] reported that Streptomyces species can degrade the aflatoxins B1 and reduce the growth pattern of the aflatoxigenic *Aspergillus flavus*.

Similarly, the *Bacillus licheniformis* has also been reported for its biodegradation potential of aflatoxins

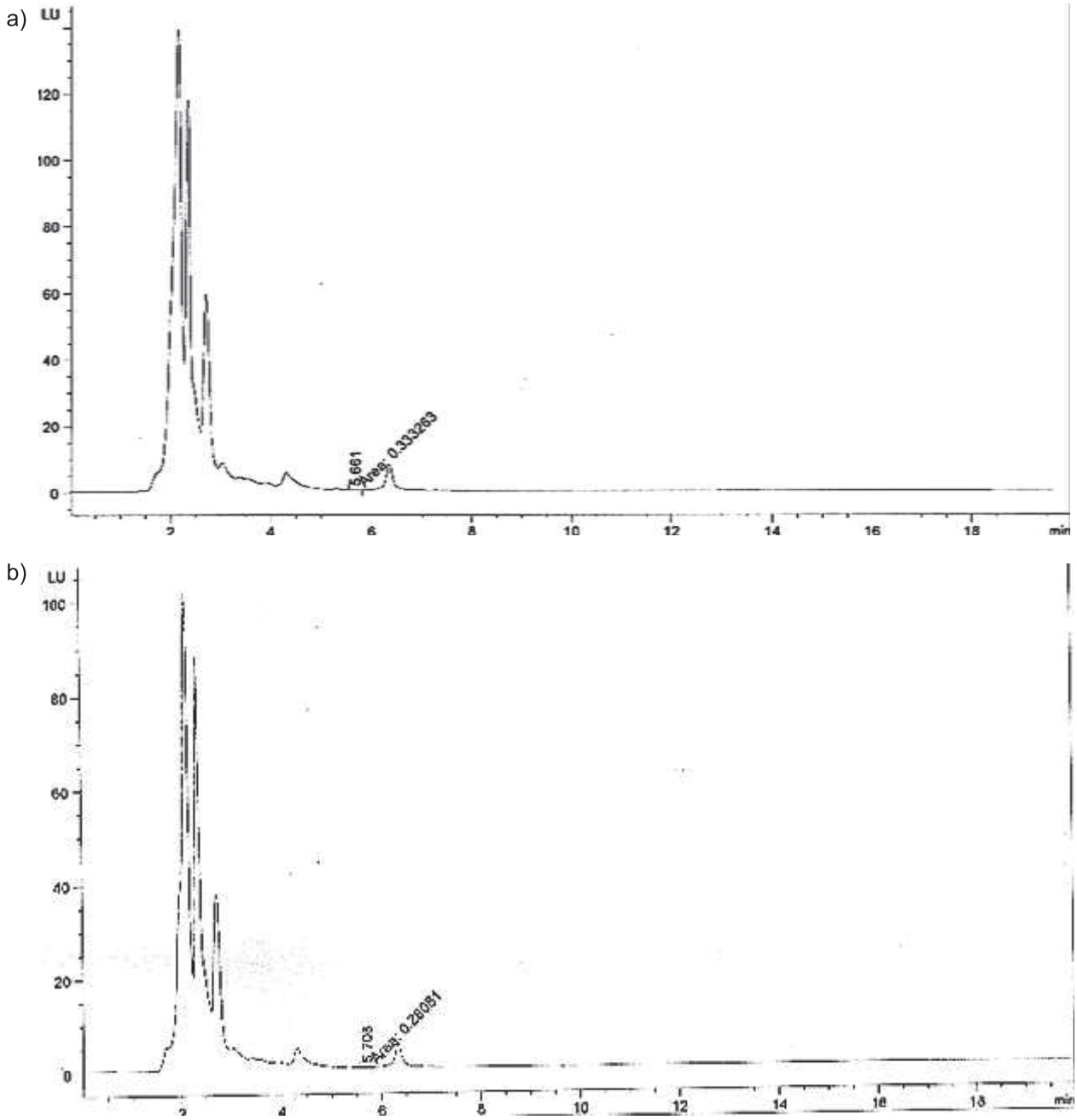

Fig. 1. a) HPLC chromatograph of biodegraded aflatoxin by *Rhodococcus erythropolis;* b) HPLC chromatograph of degraded aflatoxin by *Bacillus licheniformis*.

[15]. In another study Wang et al. [17] reported 89.1% reduction of aflatoxins B1 by *Bacillus licheniformis* within 120 h of incubation. In another study it has been reported that *Escherichia coli* CG1061 isolated from chicken cecum showed 93.7% potential of degradation aflatoxins B1 [18]. Adebo et al. [19] successfully used *Staphylococcus warneri*, Sporosarcina sp. and *Lysinibacillus fusiformis* for the biodegradation and detoxification of aflatoxins B1. In a similar study *Bacillus velezensis* DY3108 were found degrading 91.5% of the aflatoxins B1 used in the test [20]. The statistical analysis shows the significant difference ($P<0.05$) between the aflatoxin pure stranded (positive control) and *Rhodococcus erythropolis* and *Bacillus licheniformis*

It was concluded that the biodegradation potential of two bacterial species namely *Rhodococcus erythropolis* and *Bacillus licheniformis* can degrade the aflatoxins B1 used in the study. This biodegradation potential of these microbes can be exploited for the reduction of aflatoxins in different feed and food products. There is no known pathogenic history of these two microbes used in the study, thus can be used for an environment friendly and biocontrol of aflatoxins.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

The authors acknowledge the financial support of ALP-PARC for the research work.

References

1. MAJEED F.A., AKBAR A., SHAFEE M., SADIA H., IQBAL S., PHADUNGCHOB B., KHAN G., KHAN S.A. Detection of Mycotoxigenic Fungi and Mycotoxins in Poultry Feed of Balochistan Pakistan. Journal of the Hellenic Veterinary Medical Society, **72**, 2741, **2021**.
2. MAJEED A.F., AKBAR A. Fungal Biodiversity of Foods and Feeds. Environmental Pollution, Biodiversity, and Sustainable Development. Apple Academic Press, 215, **2020**.
3. ABDEL-SHAFI S., SHEHATA S., SHINDIA A., EL-MELIGY K., KHIDR A. Biodegradation of Aflatoxins by bacteria. Egyptian Journal of Microbiology, **53**, 241, **2018**.
4. SMITH M-C., MADEC S., COTON E., HYMERY N. Natural co-occurrence of mycotoxins in foods and feeds and their in vitro combined toxicological effects. Toxins, **8**, 94, **2016**.
5. KHAN G.I., AKBAR A., ANWAR M., SHAFEE M., SADIA H., RAZZAQ A., REHMAN F.U. 01. Eco-friendly bacterial biodegradation of mycotoxins. Pure and Applied Biology (PAB), **9**, 1708, **2020**.
6. JI C., FAN Y., ZHAO L. Review on biological degradation of mycotoxins. Animal Nutrition, **2**, 127, **2016**.
7. VANHOUTTE I., AUDENAERT K., DE GELDER L. Biodegradation of mycotoxins: Tales from known and unexplored worlds. Frontiers in microbiology, **7**, 561, **2016**.
8. AKBAR A., KHAN M.I., KHAN G.I. Probiotics in Biodegradation of Microbial Toxins: Principles and Mechanisms. Probiotics, Prebiotics and Synbiotics: Technological Advancements Towards Safety and Industrial Applications, 161, **2022**.
9. HALASZ A., LASZTITY R., ABONYI T., BATA A. Decontamination of mycotoxin-containing food and feed by biodegradation. Food Reviews International, **25**, 284, **2009**.
10. AKBAR A., MAJEED F.A., SADIQ M.B., KHAN S.A., RABAAN A.A. Mycotoxins occurrence in food commodities, their associated hazards and control strategies. Journal of the Hellenic Veterinary Medical Society, **73**, 3853, **2022**.
11. AFSHAR P., SHOKRZADEH M., RAEISI S.N., GHORBANI-HASANSARAEI A., NASIRAII L.R. Aflatoxins biodetoxification strategies based on probiotic bacteria. Toxicon, **178**, 50, **2020**.
12. AMAN ULLAH H., DURRANI A., IJAZ M., JAVEED A. Aflatoxin B1 contamination status of concentrate feeds of dairy goats in Lahore, Pakistan. Sarhad J Agric, **32**, 57, **2016**.
13. CSERHÁTI M., KRISZT B., KRIFATON C., SZOBOSZLAY S., HÁHN J., TÓTH S., NAGY I., KUKOLYA J. Mycotoxin-degradation profile of Rhodococcus strains. International Journal of Food Microbiology, **166**, 176, **2013**.
14. KRIFATON C., KRISZT B., SZOBOSZLAY S., CSERHÁTI M., SZŰCS Á., KUKOLYA J. Analysis of aflatoxin-B1-degrading microbes by use of a combined toxicity-profiling method. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, **726**, 1, **2011**.
15. VERHEECKE C., LIBOZ T., MATHIEU F. Microbial degradation of aflatoxin B1: current status and future advances. International Journal of Food Microbiology, **237**, 1, **2016**.
16. CAMPOS-AVELAR I., COLAS DE LA NOUE A., DURAND N., CAZALS G., MARTINEZ V., STRUB C., FONTANA A., SCHORR-GALINDO S. Aspergillus flavus Growth Inhibition and Aflatoxin B1 Decontamination by Streptomyces Isolates and Their Metabolites. Toxins, **13**, 340, **2021**.
17. WANG Y., ZHANG H., YAN H., YIN C., LIU Y., XU Q., LIU X., ZHANG Z. Effective biodegradation of aflatoxin B1 using the Bacillus licheniformis (BL010) strain. Toxins, **10**, 497, **2018**.
18. WANG L., WU J., LIU Z., SHI Y., LIU J., XU X., HAO S., MU P., DENG F., DENG Y. Aflatoxin B1 Degradation and Detoxification by Escherichia coli CG1061 Isolated From Chicken Cecum. Frontiers in Pharmacology, **9**, **2019**. Doi:10.3389/fphar.2018.01548
19. ADEBO O.A., NJOBEH P.B., MAVUMENGWANA V. Degradation and detoxification of AFB1 by Staphylococcoccus warneri, *Sporosarcina* sp. and Lysinibacillus fusiformis. Food Control, **68**, 92, **2016**.
20. SHU X., WANG Y., ZHOU Q., LI M., HU H., MA Y., CHEN X., NI J., ZHAO W., HUANG S. Biological degradation of aflatoxin B1 by cell-free extracts of Bacillus velezensis DY3108 with broad PH stability and excellent thermostability. Toxins, **10**, 330, **2018**.