The Quarterly Scientific Journal of Applied Biology
Vol.35, No.1, Ser.70, Spring 2022, P: 1-4
Journal homepage: https://jab.alzahra.ac.ir
10.22051/JAB.2021.34043.1392

Research Paper

In vitro evaluation of the effect of the organic adsorbent on the reduction of mycotoxin zearalenone

F. Adami Ghamsari[1],[*] M. Tajabadi Ebrahimi[2], M. Bagheri Varzaneh[3], A. Iranbakhsh[1], A. Akhavan Sepahi [4]

Dept. of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran *Corresponding author. Email: M.tajabadi@iauctb.ac.ir

Dept. of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran

Dept. of Agriculture, Iranian Research Organization for Science and Technology, Tehran, Iran

Dept. of Microbiology, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Teheran, Iran

Received: 2021.03. 12 **Accepted: 2021.08.24**

Zearalenone (ZEA) is one of the most important and abundant fungal toxins contaminants in food and feed that causes severe and dangerous damage to humans and livestock (Kemboi et al., 2020). This mycotoxin possesses intense estrogenic activity and causes side effects including reduced feed intake, decreased milk production, increased somatic cell count (SCC), mammary gland swelling, abortion, stillbirth, reductionin sperm quality, and infertility in animals, especially livestock (Coppock et al., 1990; Towers et al., 1995; Whitlow, 2005; Gaskill, 2008).

Given the ability of lactic acid bacteria (LAB) to inhibit the growth of some fungi and to adsorb and remove several mycotoxins such as DON and aflatoxin, these bacteria can be good candidates for bio-control of fungal contaminated feed (Franco et al., 2011). In the present study, the ability of *Lactobacillus* strains to reduce the mycotoxins ZEA was evaluated by employing in vitro approaches.

Materials & Methods

One gram of the frozen stock containing each of the four *Lactobacillus* strains including *Lactobacillus brevis* TD4 (IBRC-10790), *Lactobacillus brevis* TD10 (IBRC-10781)), *Lactobacillus casei* T2 (IBRC-10783), and *Lactobacillus paracasei* TD3 (IBRC-10784) was separately activated in 15 ml of MRS broth medium for 24 hr at 37 °C. To examine the adsorption of active bacteria, 1 ml of the culture of each bacterium was inoculated separately into 10 ml of MRS broth culture medium. Meanwhile, 10 ml of the activated culture of each bacterium was centrifuged separately. To prepare the heated bacteria, the cell pellets of each bacterium were autoclaved (at 121 °C for 20 minutes in 1 atmosphere) and were reached ambient temperature. Next, the cell pellets of heated and unheated of each bacterium were added to 10 ml of saline phosphate buffer solution (PBS). Meanwhile, a suspension of the yeast cell wall with a final concentration of 150 g/ml was made in PBS solution. Next, the ZEA mycotoxin with an initial concentration of 500 and 1000 ppb was added separately to all of the above samples in which the final concentration of bacteria was 10^9 CFU/ml in all samples (Karlovsky et al., 2016). The sterile PBS solution containing the above-mentioned concentrations of mycotoxin was considered as a control. After 0, 24, and 72 hr incubation in a shaker incubator (37 °C, 120 rpm), the samples were centrifuged (14000 × g, 20 min, 5 °C). Finally, after passing the supernatant through a 0.22 µm syringe filter the amount of remained toxin was measured by HPLC. Two strains of *Lactobacillus* with the highest adsorption in PBS solution and MRS broth medium were selected separately to analyze their synergistic effect on the reduction of ZEA toxin.

The HPLC system was equipped with Gilson 151 UV-Vis detector and a C18 column (250 × 4.6 mm, 5 °C). The obtained data was analyzed employing Gilson 712 software. The mobile phase consisted of a mixture of acetonitrile and double distilled water with a ratio of 55:45 (v/v). The device was set in isocratic mode with a flow rate of 1 ml/min and the temperature of the column was 30 °C. An injection volume of 50 µl was chosen and the optical density of ZEA was determined at 336 nm. The GraphPad Prism 6 software was used for statistical analysis and the comparisons between means ($P < 0.05$) were determined using an unpaired t-test and analysis of variance (ANOVA).

Results & Discussion

The control and removal of the fungal contamination from livestock and poultry food represents very necessary issues worldwide. For this purpose, the biological method, particularly, the use of organisms can be appropriate and effective. The most important components of the cell wall of lactic acid bacteria include peptidoglycan chains, polysaccharides, and proteins which are mainly responsible for the adhesion of lactic acid bacteria to toxins. The carbohydrates, especially glucan, polysaccharides, peptidoglycans, proteins, teichoic acid, as well as hydrophobic segments of the cell wall of lactic acid bacteria possess the binding potential to the mycotoxins (Niderkorn et al., 2009; Piotrowska, 2014).

The results showed that *Lactobacillus* strains and yeast cell walls were able to adsorb and reduce mycotoxin ZEA, significantly ($P < 0.05$). The mycotoxin adsorption was increased over incubation time and in comparison to the twice concentration of ZEA, *Lactobacillus brevis* TD4 adsorped a higher amount of ZEA when its initial concentration was 500 ppm, although they were not significant. The organic adsorbents investigated in the two states heated in PBS solution and active in the MRS broth culture medium possessed a higher adsorption rate than the non-heated state in PBS solution.

Deactivation by heat causes a considerable impact on the ability of lactic acid bacteria to remove mycotoxins. The cell wall peptidoglycan of lactic acid bacteria, such as *Lactobacillus* has in vitro the ability to connect and reduce the ZEA (Niderkorn et al., 2009). *Lactobacillus paracasei* isolated from the starter culture of sour paste detoxified toxins by surface binding and enzymatic decomposition from the broth culture (Hassan et al., 2015). Manoproteins, glucans, and proteins on the outer layer of the yeast cell wall are involved in binding to mycotoxins. Differences in cell wall carbohydrates and chitin content between yeast strains as well as the equilibrium between polar and hydrophobic groups in the yeast cell wall and mycotoxin are significant in binding affinity (Niderkorn et al., 2009). Also, the three-dimensional structure of yeast cell wall components especially D-glucans, the geometric molecular similarity of binder and toxin, and hydrophobic interactions between glucose units in a single helix in D-glucans and ZEA represent key factors in adsorption efficiency (Freimund et al., 2003).

By considering various parameters, the synergism effects of *L. brevis* strain TD4 and *L. paracasei* strain TD3 showed a higher rate of toxin reduction than the each of strains alone. The correlation coefficient (R2) of mycotoxin ZEA was $R2 = 0.9392$ and the recovery range

was 85%-110%. The relative standard deviation (RSD) of ZEA mycotoxin on one day and three consecutive days was calculated to be 3.05% and 5.5%, respectively. The amount of estimated LOQ and LOD was 116.13 ppb and 38.32 ppb, respectively.

Conclusion:

The present study investigated the adsorption and reduction of the mycotoxin zearalenone by the organic adsorbent containing four native Iranian isolated from dairy fermented products and the yeast cell wall of *Saccharomyces cerevisiae*. By employing the HPLC method, the effect of different parameters and synergy of strains on mycotoxin adsorption and reduction was examined. Therefore, the use of the studied organic adsorbent can be effective in reducing and controlling the contamination of zearalenone mycotoxin.

Keywords: *Adsorbent, Lactobacillus, Mycotoxin, Probiotic, Yeast cell wall, Zearalenone*

Acknowledgement*:*

This article is extracted from the approved and defended microbiology Ph.D. thesis of Fatemeh Adami Ghamsari in the Faculty of Basic Sciences of the Islamic Azad University, Science and Research Branch, Tehran, Iran. The authors would like to thank Dr. Abdolamir Ghadaksaz for his helpful assistance and support.

Declaration of conflict of interest:

The authors declare that there is no conflict of interest.