IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-14, Issue4, 2023, pp01-07
www.bipublication.com

**Review Article**

# How will the industry innovate to solve the problem of mycotoxins in biopharmaceutical production?

**Nicoly Subtil de Oliveira, Rafaela Caldas de Paula, AngelaBonjorno Arantes, Luiz Fernando Bianchini and Edvaldo Antonio Ribeiro Rosa***

Pontifícia Universidade Católica do Paraná.
Xenobiotics Research Unit, Curitiba, Brazil

*Corresponding author: Prof. Edvaldo Antonio Ribeiro Rosa
Pontifícia Universidade Católica do Paraná. School of Medicine and Life Sciences. Xenobiotics Research Unit. RuaImaculadaConceição 1155. Zip 80250-901, Curitiba, Brazil. Phone: +55 41 3271-1497.
Email: edvaldo.rosa@pucpr.br

[Received 09 May-2023, Accepted 23 Sept-2023, Published 01 Oct -2023]

**Abstract**

The Biopharmaceuticals market is going to reach near USD 1 trillion/year. Fungi are suitable sources of secondary metabolites that act as biodrugs and may conduce biotransformations that convert old drugs into new ones. The problem that arises with that is how to eliminate a group of serious contaminants of fungal fermentations – mycotoxins. In this mini-review, we present some resources' limitations and an alternative to industry to solve this problem, using an innovative approach.

**Keywords:** Biopharmaceuticals, Mycotoxins, Fungal fermentation, Mitigation

## 1. Introduction

The global biopharmaceuticals market grows fast and was valued at about USD 291.4 billion in 2020, with an expected rate of reaching USD 974.5 billion in 2030 [20]. This category of drugs includes vaccines, hyper-immune serums, blood products, biopharmaceuticals obtained from biological fluids or tissues of animal origin and/or obtained by biotechnological procedures, monoclonal antibodies, and drugs containing live, attenuated, or dead microorganisms [28]. Some studies also found a significant decrease in incidence of hypotension in group of ephedrine use.[17]

Microbial secondary metabolites, such as antibiotics and therapeutic enzymes (e.g., L-asparaginase, nattokinase), and biotransformation products (e.g., steroids) can be produced by submerged fermentation employing filamentous fungi and are considered biopharmaceuticals.

The most used fungal genera in these bioprocesses are *Penicillium*, *Aspergillus*, *Fusarium*, *Gibberella*, and *Trichoderma*[30]; notably known to produce mycotoxins of diverse nature [1].

Mycotoxins are very heterogeneous metabolites both chemically and toxicologically buthave low molar masses

as a common feature. Although there are hundreds of types of mycotoxins, the main classes are aflatoxins (B1, B2, G1, and M1), fumonisins (B1 and B2), ochratoxin A, ergotamine, zearalerone, citrinin, patulin, trichothecenes type A (neosolaniol), type B (nivalenol, deoxynivalenol, and trichothecin), type C (crotocin), and type D (atranones, verrucarins A and B, roridine A, and satratoxin H).

Mycotoxins form the largest group of contaminants in animal feed, food industry ingredients, and finished food products. They are toxic secondary metabolites (hence not essential for cell development) that are produced primarily to protect the fungi against other organisms competing for the same niche and resources [9,31,]. They can also act as cellular performance enhancers, as they increase phytotoxicity, favouring colonization [37], in addition to protecting them from oxidative stress [32,33] and predation by fungivorous arthropods [34].

Illnesses caused by mycotoxins involve debilitating neurological, cognitive, or hormonal impairment. More specifically, they can cause various respiratory problems, elevation of cholesterol, problems with haemostasis, increase in urinary frequency and volume, gain or loss of body mass, various endocrine disorders (diabetes, reduction in testosterone levels, pubertal precocity), autoimmune disorders, reactivation of dormant viruses, dizziness, tremors, fatigue, apathy, depression, anxiety, memory problems, abdominal pain, diarrhoea, development of celiac disease, weakness, joint problems, muscle pain, among others.

## 2. The problem called mycotoxins

About bioprocesses, mycotoxins can be secreted into the liquid (SmF) or solid (SSF) substrate and contaminate the bulk of enzymes or other metabolites of interest (e.g., antibiotics, antitumor, immunosuppressant, pigments, antioxidants, biotransformed products etc.). Such chemical contamination demands dedicated technology that raises production costs considerably.

The problem of mycotoxin co-expression during fermentation processes is usually overcome when the biopharmaceutical is of protein origin (e.g., L-asparaginase) or derived from cellular content (e.g., β-glucans). In these situations, selective precipitation and multiple washes can purify the product of interest. However, when the drug is of low molar mass or derived from fungal biotransformation, there is a risk of mixture with mycotoxins diluted in the bulk, deserving more attention.

## 3. Primary solutions

Rational approaches for mitigating the problem are the use of industrial strains that do not produce such substances, as occurs with several zygomycetes [24], or strains that contain large DNA deletions in the regions where the associated genes are found [4]. Another option is to generate disabling mutations in these genes [10]. Although these are very interesting strategies, they demand a lot of laboratory effort and several mycotoxin-producing species are superior in the production of molecules of interest [19,18].

If this approach is chosen, continuous monitoring of the appearance of mycotoxins is mandatory, which can occur by genetic reversal, suppression of expression silencing, or even contamination. In this case, there is the possibility of losing batches in which the presence of mycotoxins was positive. A good example of monitoring is that carried out by the British company Quorn Foods, which produces mycotoxin-free edible mycoprotein from *Fusarium venenatum*.

A strategy that has been receiving attention is the detoxification of mycotoxins present in the bulk by accessory microbial biotransformation. This feature includes

acetylation, glycosylation, ring breaking, hydrolysis, deamination and decarboxylation reactions performed by other microorganisms that are added to the bulk to neutralize mycotoxins. Hathout and Aly [13] compiled and reviewed several studies involving bacteria from different phyla and filamentous fungi and yeasts that, if added to certain mycotoxins, can detoxify them with a varied efficacy. However, if this practice proves to be viable for consumer goods that are not attacked by microorganisms during detoxifying biotransformation processes (e.g., for food and beverages), in the production of biopharmaceuticals, most likely, the molecules of interest would also be bioconverted, with substantial losses.

In practice, current mycotoxin removal techniques involve binding and adsorption, which are dependent on the physicochemical parameters of the molecules (polarity, solubility, size, three-dimensional conformation, charge distribution, and dissociation constant)[15], the crystalline structures of the adsorbent material [6], and the physical conditions existing during adsorption (temperature, pH, salinity).

The main used agents are aluminosilicates as natural or activated zeolites and bentonites [23]. This is a known and widely explored reality in animal nutrition, but very little is applied in the production of biopharmaceuticals.

Aluminosilicate is a generic term comprising inert clays that adsorb mycotoxins over a wide pH range. Several minerals, such as albite, calcite, clinoptilolite, dolomite, montmorillonite, sepiolite, smectite, and thenardite, are aluminosilicates widely used in animal nutrition [7]. These minerals are naturally occurring, inexpensive, and easily modified to increase mycotoxin binding capacity [14]. However, from an industrial point of view, these minerals, as well as activated carbon and kaolin, can also eliminate other molecules[27], including biopharmaceuticals to be purified.

**4. Rational and innovative solutions**

Another approach for the recovery of fungal biopharmaceuticals, which in our view is more feasible, involves the use of cellular components such as β-glucans and peptidoglycans. Reports show the high efficiency of β-glucans in removing different classes of mycotoxins [25,8,11].

β-glucans are biologically active polysaccharides that are part of the cell wall of cereals, bacteria, and fungi and that contain only glucose as a monomeric unit [35].

Organic adsorbents such as yeast β-glucans have a very different mycotoxin neutralization mechanism and characteristics from those of clay minerals. The high adsorption affinity and the low dissociation of mycotoxins by intact yeast cells or cell wall fractions were observed, even at low pH values [12]. While mycotoxins bind to inorganic clays via ionic bonds with hydroxyl or carbonyl radicals, β-glucans allow the establishment of hydrogen bonds and van der Waals forces, characterizing the interaction by adsorption and not by bonding.

The reticular organization of β-glucans and the distribution of β-(1,3)-D-glucans and β-(1,6)-D-glucans resulting from different production processes interfere in the affinity for different mycotoxins[38]. Alkaline extractions of β-glucans disrupt the molecular architecture, resulting in lower adsorption rates. On the other hand, cell walls kept intact and with unaltered spatial structure show superior performance [12,26].

Furthermore, β-glucans obtained from different genera of yeasts may prove to be efficient in removing mycotoxins [36,2].

Peptidoglycans from Gram-positive bacteria can bind to mycotoxins in an effective manner and have aroused interest in their use [16]. Its ability to bind free toxins is due

to hydrophobic interactions [3] with peptide moieties[22]. Aflatoxin B1, deoxynivalenol, fumonisins, zearalenone, and other mycotoxins can be appreciably reduced in the presence of this material [21,3].

The removal of mycotoxins in the liquid phase has been evaluated in fluid matrices such as milk [25,5] and liquid animal feed [29], with promising results. In these studies, neutralizing agents were added to the liquid matrices and remained under agitation. In the case of biopharmaceutical purification, it would be much more practical to use affinity columns packed with the adsorbent material, which would maximize the chances of removal.

An interesting finding was that both β-glucans and peptidoglycans removed mycotoxins but did not affect the content of other molecules [25], which is interesting when purifying biopharmaceuticals.

It should be noted that these mycotoxin adsorption approaches, although effective in reducing, do not promote the complete elimination of these substances from the liquid bulk. This is due to the methodology used in the studies, mostly based on the mere mixture of agents under agitation and on the specificity of the absorbents. The attempt to obtain a "universal binder" must contemplate a mixture of absorbents.

## 5. Conclusion

In summary, the production of biopharmaceuticals by fermentative processes using fungi can lead to the undesired co-production of mycotoxins, which must be removed from the liquid bulk, before continuing their downstream processing. The passage of this bulk through columns packed with mixtures of adsorbents should be the best strategy for minimizing mycotoxin concentrations.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Acknowledgments

"This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001"

## References

1. Bennett, J.W., Klich, M. 2003. Mycotoxins. Clin. Microbiol. Rev. 16,497-516. https://doi.10.1128/CMR.16.3.497-516.2003.
2. Bzducha-Wróbel, A., Bryła, M.,Gientka, I., et al. 2019. *Candida utilis* ATCC 9950 cell walls and β(1,3)/(1,6)-glucan preparations produced using agro-waste as a mycotoxins trap. Toxins (Basel).11,192. https://doi:10.3390/toxins11040192.
3. Chlebicz, A.,Śliżewska, K. 2020. In vitro detoxification of aflatoxin B1, deoxynivalenol, fumonisins, t-2 toxin and zearalenone by probiotic bacteria from genus *Lactobacillus* and *Saccharomyces cerevisiae* yeast. Probiotics Antimicrob. Proteins.12,289-301. https://doi:10.1007/s12602-018-9512-x.
4. Christensen, B.E., Mølgaard, H., Kaasgaard, S., et al.2000.Methods for producing polypeptides in *Aspergillus* mutant cells. Patent WO2000039322A1, published2000 July 6.
5. Corassin, CH., Bovo, F., Rosim, R.E., et al. 2013. Efficiency of *Saccharomyces cerevisiae* and lactic acid bacteria strains to bind aflatoxin M1 in UHT skim milk. Food Control.31,80-83.https://doi.org/10.1016/j.foodcont.2012.09.033.
6. Daković, A., Tomašević-Čanović, M., Dondur, V., et al. 2005. Adsorption of mycotoxins by organozeolites. Colloids Surf. B Biointerfaces.46,20-25. https://doi:10.1016/j.colsurfb.2005.08.013.
7. De Mil, T., Devreese, M., De Baere, S., et al. 2015. Characterization of 27

mycotoxin binders and the relation with in vitro zearalenone adsorption at a single concentration. Toxins (Basel).7,21-33. https://doi:10.3390/toxins7010021.

8. El-Naggar, M.A.,Thabit, T.M. 2014. Evaluation of β-D-glucan biopolymer as a novel mycotoxin binder for fumonisin and deoxynivalenol in soybean feed. Foodborne Pathog. Disease.11,433-438. https://doi:10.1089/fpd.2013.1711.
9. Fox, E.M., Howlett, B.J. 2008. Secondary metabolism: regulation and role in fungal biology. Curr.Opin.Microbiol.11,481-487. https://doi:10.1016/j.mib.2008.10.007.
10. Frisvad, J.C., Møller, L.L.H., Larsen, T.O., et al. 2018. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*. Appl. Microbiol.Biotechnol. 102,9481-9515. https://doi:10.1007/s00253-018-9354-1.
11. Fruhauf, S., Schwartz, H., Ottner, F., et al. 2012. Yeast cell based feed additives: studies on aflatoxin $B_1$ and zearalenone. Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess. 29,217-231.https://doi:10.1080/19440049.2011.630679.
12. Han, L. 2021. What can contribute to a higher adsorption affinity of YCW? PigProgress. https://www.pigprogress.net/specials/what-can-contribute-to-a-higher-adsorption-affinity-of-ycw (accessed 5 September 2022).
13. Hathout, A.S,Aly, S.E. 2014. Biological detoxification of mycotoxins: a review. Ann. Microbiol.64,905–919.https://doi.org/10.1007/s13213-014-0899-7.
14. Horky, P., Gruberova, H.A., Aulichova, T., et al. 2021. Protective effect of a new generation of activated and purified bentonite in combination with yeast and phytogenic substances on mycotoxin challenge in pigs. PLoS One.16,e0259132. https://doi:10.1371/journal.pone.0259132.
15. Huwig, A., Freimund, S., Käppeli, O., et al. 2001. Mycotoxin detoxication of animal feed by different adsorbents. Toxicol. Lett.122,179-188. https://doi:10.1016/s0378-4274(01)00360-5.
16. Liew, W.-P.-P., Nurul-Adilah, Z., Than,et al. 2018. The binding efficiency and interaction of *Lactobacillus casei* Shirota toward aflatoxin B1. Front. Microbiol.10,9. https://doi:10.3389/fmicb.2018.01503.
17. Muhammad Fahad Ismail, Ayesha Altaf, & Asad Ahmad. (2021). A Comparative Study on Therapeutic modalities in the Management of Spinal Anesthesia-Induced Hypotension in Parturient in Cesarean Delivery. *International Journal of Advances in Health Sciences (IJHS)*, *8*(4), 5–10. Retrieved from https://www.ijhsonline.com/index.php/IJHS/article/view/226
18. Meyer, V., Andersen, M.R., Brakhage, A.A., et al. 2016. Current challenges of research on filamentous fungi in relation to human welfare and a sustainable bio-economy: a white paper. Fungal Biol.Biotechnol.3,6. https://doi:10.1186/s40694-016-0024-8.
19. Meyer, V., Basenko, E.Y., Benz, J.P., et al. 2020. Growing a circular economy with fungal biotechnology: a white paper. Fungal Biol.Biotechnol.7,5. https://doi:10.1186/s40694-020-00095-z.
20. Mikulic, M. 2022. Biopharmaceuticals market size worldwide forecast 2020-2030. https://www.statista.com/statistics/1293077/global-biopharmaceuticals-market-size (accessed 3 September 2022).
21. Nasrollahzadeh, A., Mokhtari, S, Khomeiri, M., et al. 2022. Mycotoxin

detoxification of food by lactic acid bacteria. Int. J. Food Contamin. 9,1.https://doi.org/10.1186/s40550-021-00087-w.

22. Niderkorn, V., Morgavi, D.P., Aboab, B., et al. 2009. Cell wall component and mycotoxin moieties involved in the binding of fumonisin B1 and B2 by lactic acid bacteria. J.Appl.Microbio.106,977-985. https://doi:10.1111/j.1365-2672.2008.04065.x.
23. Papaioannou, D., Katsoulos, P. D., Panousis, N.,et al. 2005.The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: A review. Microporous Mesoporous Mater. 84,161-170. https://doi:10.1016/j.micromeso.2005.05.030.
24. Partida-Martinez, L.P., de Looss, C.F., Ishida, K., et al. 2007. Rhizonin, the first mycotoxin isolated from the zygomycota, is not a fungal metabolite but is produced by bacterial endosymbionts. Appl. Environ.Microbiol. 73,793-797. https://doi:10.1128/AEM.01784-06.
25. Pavlek, Ž., Bošnir, J., Ivešić, M., et al. 2021. Investigation of milk quality after removal of AFM1 using lactic acid bacteria and beta-glucan. Medica Jadertina.51,5-12.https://hrcak.srce.hr/256558.
26. Piotrowska, M.,Masek, A. 2015.*Saccharomyces cerevisiae* cell wall components as tools for ochratoxin a decontamination. Toxins (Basel).7,1151-1162. https://doi:10.3390/toxins7041151.
27. Piotrowska, M. 2021. Microbiological decontamination of mycotoxins: opportunities and limitations. Toxins (Basel). 13,819. https://doi:10.3390/toxins13110819.
28. Pour, A.H.A., Rahmani, F., Shams-Ghahfarokhi, M., et al. 2021. Fungal biopharmaceuticals: current research, production, and potential applications. In: Industrially important fungi for sustainable development. In: Abdel-Azeem, A.M., Yadav, A.N., Yadav, N., Sharma, M. (eds), Fungal biology,Cham: Springer.
29. Ragoubi, C., Quintieri, L., Greco, D., et al. 2021.Mycotoxin removal by *Lactobacillus* spp. and their application in animal liquid feed. Toxins (Basel). 13,185. https://doi:10.3390/toxins13030185.
30. Raja, H.A., Miller, A.N., Pearce, C.J.,et al. 2017. Fungal identification using molecular tools: a primer for the natural products research community. J. Nat. Prod.80,756-770. https://doi:10.1021/acs.jnatprod.6b01085
31. Rasmussen, T.B., Skindersoe, M.E., Bjarnsholt, T., et al. 2005. Identity and effects of quorum-sensing inhibitors produced by *Penicillium* species. Microbiology (Reading). 151,1325-1340. https://doi:10.1099/mic.0.27715-0.
32. Reverberi, M., Zjalic, S., Ricelli, A., et al. 2006. Oxidant/antioxidant balance in *Aspergillus parasiticus* affects aflatoxin biosynthesis. Mycotoxin Res.22,39-47. https://doi:10.1007/BF02954556.
33. Reverberi, M., Zjalic, S., Ricelli, A., et al. 2008. Modulation of antioxidant defense in *Aspergillus parasiticus* is involved in aflatoxin biosynthesis: a role for the ApyapA gene. Eukaryot. Cell.7,988-1000. https://doi:10.1128/EC.00228-07.
34. Rohlfs, M., Albert, M., Keller, N. P.,et al. 2007. Secondary chemicals protect mould from fungivory. Biol. Lett.3,523-525. https://doi:10.1098/rsbl.2007.0338.
35. Rop, O., Micek, J., Jurikova, T. 2009. Beta-glucans in higher fungi and their health effects. Nutr. Rev.67,624–631. https://doi:10.1111/j.1753-4887.2009.00230.x.
36. Tapingkae, W., Srinual, O., Lumsangkul, C., et al. 2022. Industrial-

scale production of mycotoxin binder from the red yeast *Sporidiobolus pararoseus* KM281507. J. Fungi (Basel).8,353. https://doi:10.3390/jof8040353.

37. Venkatesh, N.,Keller, N.P. 2019. Mycotoxins in conversation with bacteria and fungi.Front.Microbiol.10,403. https://doi:10.3389/fmicb.2019.00403.
38. Yiannikouris, A., François, J., Poughon, L., et al. 2004. Adsorption of zearalenone by β-d-glucans in the *Saccharomyces cerevisiae* cell wall. J.Food Protect.67,1195–1200. https://doi:10.4315/0362-028x-67.6.1195.