

AN *IN SILICO* APPROACH FOR THE EVALUATION OF CELL WALL COMPONENTS OF PROBIOTICS AS BIOLOGICAL ADSORBERS OF AFLATOXINS

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ABSTRACT

Aflatoxins are produced in poultry feed by two major fungal species viz., Aspergillus flavus and Aspergillus parasiticus during hot and humid seasons. To detoxify these aflatoxins, various binders are being used in the field which are mostly synthetic compounds with possible other toxicities, the harmless beneficial probiotics are being considered as binders to neutralize the effect of aflatoxins in the feed. Bacterial organisms (probiotics) like Lactobacillus rhamnosus, Lactobacillus casei, Bacillus subtilis, Enterococcus faecium and yeast (Saccharomyces cerevisiae) were selected and their cell wall structures have been retrieved and molecular docking was performed against four types of aflatoxins namely AFB1, AFB2, AFG1 and AFG2. The results revealed that cell wall components of the chosen bacterial organisms have a good binding affinity towards four types of aflatoxins. Based on this in silico results, it is evident that the both the lipoteichoic acid (LTA) and wall teichoic acid (TA) in the cell wall of probiotic organisms is responsible for its binding against aflatoxins.

Keywords: Aflatoxicosis, biological binder, *in silico*, probiotics

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INTRODUCTION

Aflatoxins causes major economic problems in poultry industry (Pattison *et al.*, 2008). Aflatoxins like other mycotoxins are toxic metabolites produced primarily by filamentous fungus *Aspergillus flavus* and *Aspergillus parasiticus* on the major ingredients of poultry feed during its harvest

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and storage (Chen *et al.*, 2005). Aflatoxicosis is common problem in tropical countries like India because of the warm and humid environment. Aflatoxins are classified as Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2) based on their blue and green fluorescence under UV light (Talebi *et al.*, 2011), of which AFB1 is the most potent hepatotoxic compound and carcinogenic substance which affects both animal and human health (Sidhu *et al.*, 2009). The toxicity of aflatoxins depends on the species affected, dose and duration of exposure.

Aflatoxins causes various effects ranged from lethality when administered as a single large dose to histopathological changes in tissues when administered as smaller repeated doses even to tumour production when given for longer duration. Chronic exposure to aflatoxin is reported to cause various growth problems like reduced feed conversion efficiency, reduced body weight gain, impaired reproductive efficiency, anaemia, jaundice and immunosuppression leading to increased mortality rate (Khlangwiset *et al.*, 2011). In layers it causes fatty liver condition and thereby decreased egg production (Wu *et al.*, 2011).

To detoxify aflatoxins, physical, chemical and biological methods are being used in field condition (Tiwari *et al.*, 2016). Of different methods available, probiotics are used as non-nutrient feed additives at small quantities. Probiotics are either given singly or in combination to enrich the bacterial microflora of the gut and promote the growth

performance of broilers (Fritts *et al.*, 2000). Lactic acid producing bacteria are used as biological adsorber of mycotoxin.

In the present study, the cell wall structures of *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Bacillus subtilis*, *Enterococcus faecium* and yeast *Saccharomyces cerevisiae* were retrieved from PDB database and the binding affinity against four aflatoxins were evaluated *insilico*.

MATERIALS AND METHODS

Aflatoxins

Aflatoxin B1 is having tetrahydrocyclopenta [c] furo [3',2':4,5] furo [2,3-h] chromene skeleton with oxygen functionality at positions 1, 4 and 11. It is an aromatic ether and aromatic ketone whereas Aflatoxin B2 is having a hexahydrocyclopenta [c] furo [3',2':4,5] furo [2,3-h] chromene skeleton. Aflatoxin G1 and G2 are members of coumarins. The structure of AFB1, AFB2, AFG1 and AFG2 were obtained in SDF format from pubchem database. The pubchem identifier of each aflatoxin is given in the Table-1.

The SDF format of each aflatoxin was saved in PDB format using Biovia Discovery studio visualizer so as to be used for docking.

Cell wall structures of probiotic organisms

The cell wall structures of *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Bacillus subtilis*, *Enterococcus faecium* and yeast *Saccharomyces cerevisiae* were retrieved

as PDB file from the protein databank. The structures of the chosen bacteria and yeast and their respective PDB Ids are given in the Table -2.

Docking

The selected bacterial cell wall structures as protein targets were converted from PDB to PDBQT formats and kept ready for docking using Autodock Vina (Trott and Olson, 2010). In the same way, four aflatoxins as ligands were also prepared and saved in PDBQT formats for docking. Each aflatoxin was docked individually with the bacterial cell structures and binding affinity (kcal/mol) was recorded. The adsorbing capacity of the selected bacterial organisms against aflatoxin were assessed by the binding efficiency. Biovia Discovery studio visualizer was used to read the interaction between the bacterial cell wall structures and different types of aflatoxin and to visualise the output structures.

RESULTS AND DISCUSSION

The chemical nature of aflatoxins in terms of molecular weight, XLogP3-AA, Hydrogen Bond Acceptor and donor, and topographical polar surface area is given in the Table -3.

The chosen bacterial cell wall structures showed good binding affinity against all the four types of aflatoxins. The binding affinity of the bacterial structures against each aflatoxin is shown in the Table – 4 and the 2D

and 3D output structures are given in the Table 5. Among the chosen structures, the wall and lipo-techoic acid structures showed greater binding affinity when compared to other structures. The basal pilin SPaB structure of *Lactobacillus rhamnosus* showed binding affinity lesser than teichoic acid but greater than tertiary structure of carrier protein and adhesion protein structures in the taken bacterial organisms. The binding affinity of basal pilin SPaB structure from *Lactobacillus* against AFG1 is lesser when compared to AFB1, whereas the binding affinity of penicillin binding protein from *Enterococcus faecium* was more against AFG1 when compared to AFB1.

Aflatoxins adsorb to the enterocytes and enter the system by passive diffusion. After adsorption, aflatoxins undergo bioactivation by the cytochrome P540 oxidase system and produce highly reactive AFB1 8,9-epoxide (AFBO) which then forms adducts with DNA, RNA and protein and leads to multi systemic disorders which in turn produces drastic production losses in terms of feed intake, body weight gain and feed conversion ratio in broilers. Probiotics reduce or detoxify aflatoxins by physically adsorbing to the aflatoxins. Adsorption is a process in which the structures of probiotics bind to the aflatoxin by non-covalent weak bonds and electrostatic attraction. In this process, important structures of bacteria involved are polysaccharides, peptidoglycan and teichoic acid present in the cell wall (Mendoza *et al.*, 2009).

In this *in silico* study, it is evident that the cell wall components of probiotics are having a good binding affinity against the four types of aflatoxins. The results of this study proves that teichoic acid present in the cell wall is responsible for detoxification of aflatoxin when compared to other cell wall structures. *Saccharomyces cerevisiae*, yeast also showed comparable results to that of other bacterial cultures. This finding is in correlation with the findings that *Saccharomyces cerevisiae* showed the greatest ability to remove the AFB1 by adsorption (Pizzolitto *et al.*, 2012).

Strong evidence from *in vitro* tests are available to substantiate the findings of this study but *in vitro* adsorption is influenced by various factors like strain and concentration of bacteria, dose of toxin, temperature, pH

and hence the process is considered as fast and easily reversible (Shahin, 2007). *In vivo* studies with single probiotic against aflatoxin are available especially with *Lactobacillus* and *Bifidobacterium* (El-Nezami *et al.*, 2000).

This *in silico* study provided that the teichoic acids present in the cell wall of probiotic organisms as the mechanism of action as a biological adsorber of aflatoxins. Only limited information is available on *in vivo* studies to support the adsorption capacity of probiotics against aflatoxin. Instead of use of single probiotic organism, combination of probiotics shall be utilized in *in vivo* trails to assess the efficacy of probiotics against aflatoxins. This will help in protection of birds from harmful effects of aflatoxins and prevent economic losses to the poultry farmers.

Table 1. Pubchem identifier of each aflatoxin

S. No.	Type of Aflatoxin	Pubchem Id
1	AFB1	186907
2	AFB2	2724360
3	AFG1	14421
4	AFG2	2724362

Table 2. Structures of the chosen bacteria and yeast and their respective PDB Ids

S. No.	Cell wall structure	PDB Id	Main function
1	Crystal structure of SPaB basal pilin from <i>Lactobacillus rhamnosus</i> GG	7CBS	SPaB is essential for anchoring to the host
2	Tertiary structure of apo-D-alanyl carrier protein of <i>Lactobacillus casei</i>	1HQB	D-alanylation of lipoteichoic acid facilitates modulation of surface charge and ligand binding regulation of gram positive organism.
3	Distinct and essential morphogenic functions for wall and lipo-techoic acids in <i>Bacillus subtilis</i>	2W8D	Lipo-techoic acid is essential for cell morphogenesis and division and wall techoic acid is for elongation.
4	Crystal structure of penicillin binding protein -5(PBP-5) from ' <i>Enterococcus faecium</i> in the closed conformation	6MKJ	Penicillin-binding proteins (PBPs) are essential in forming the final cross-links in peptidoglycan chains of the bacterial cell wall.
5	Structure of the N-terminal domain of the F101 adhesin (n-flo1p) from the yeast <i>Saccharomyces cerevisiae</i>	4LHL	F101 adhesin is essential for cell-cell adhesion, conjugation and survival.

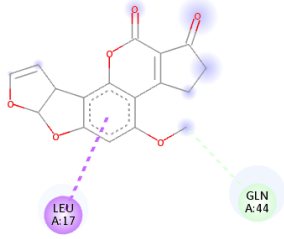
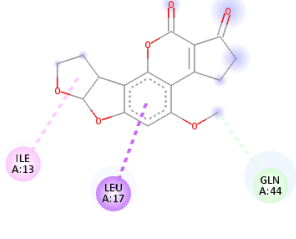
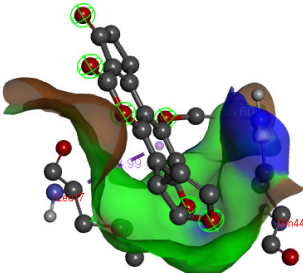
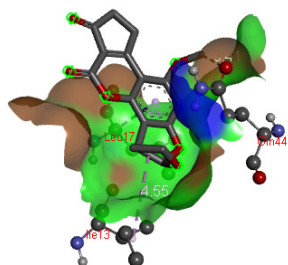
Table 3. Chemical nature of aflatoxins

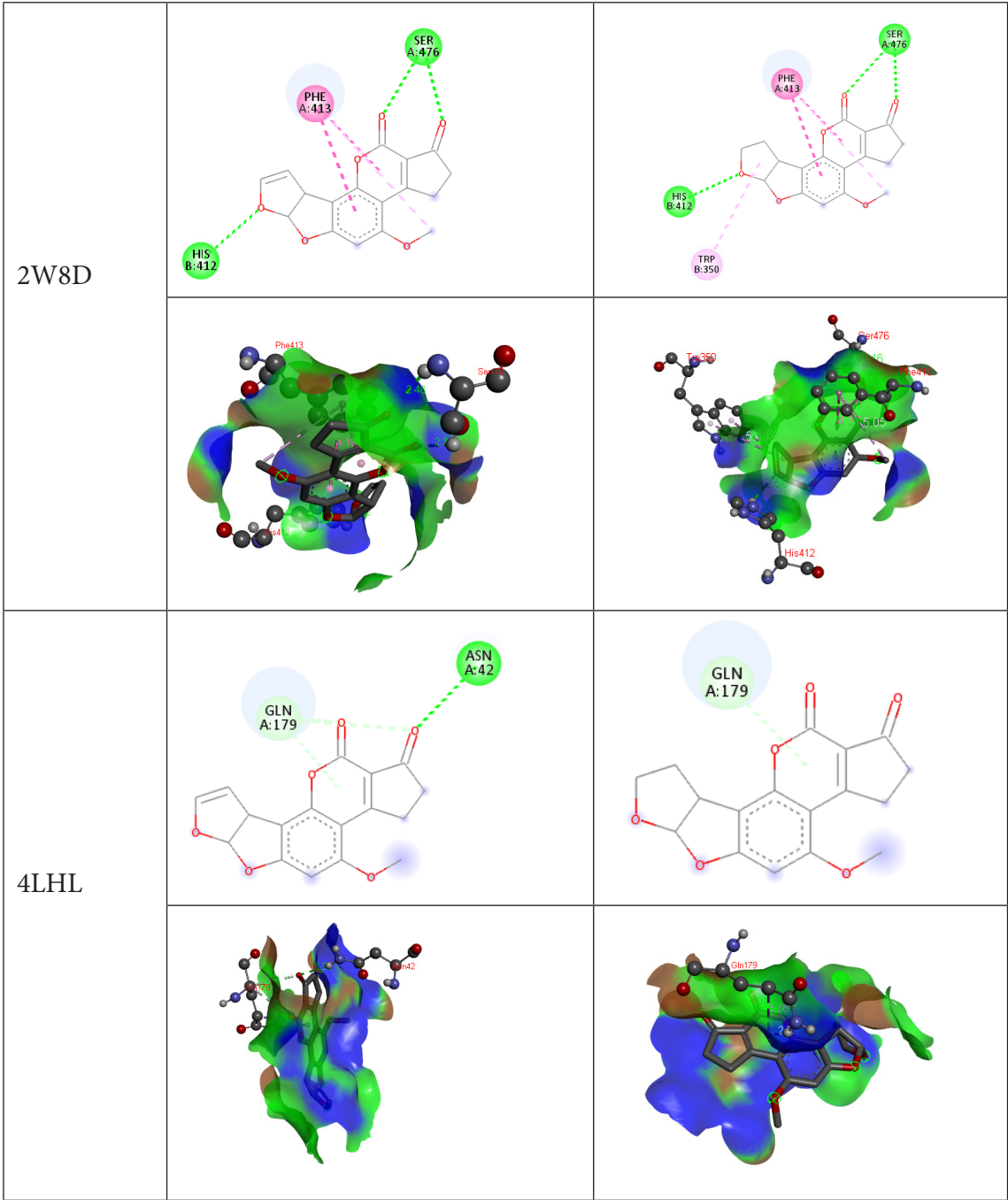
S.No.	Type of Aflatoxin	Molecular Weight	XLogP3-AA	Hydrogen Bond acceptor Count	Hydrogen Bond donor Count	Topological Polar Surface Area
1	AFB1	312.27	1.6	6	0	71.1 Å ²
2	AFB2	314.29	1.3	6	0	71.1 Å ²
3	AFG1	328.27	1.8	7	0	80.3 Å ²
4	AFG2	330.29	1.5	7	0	80.3 Å ²

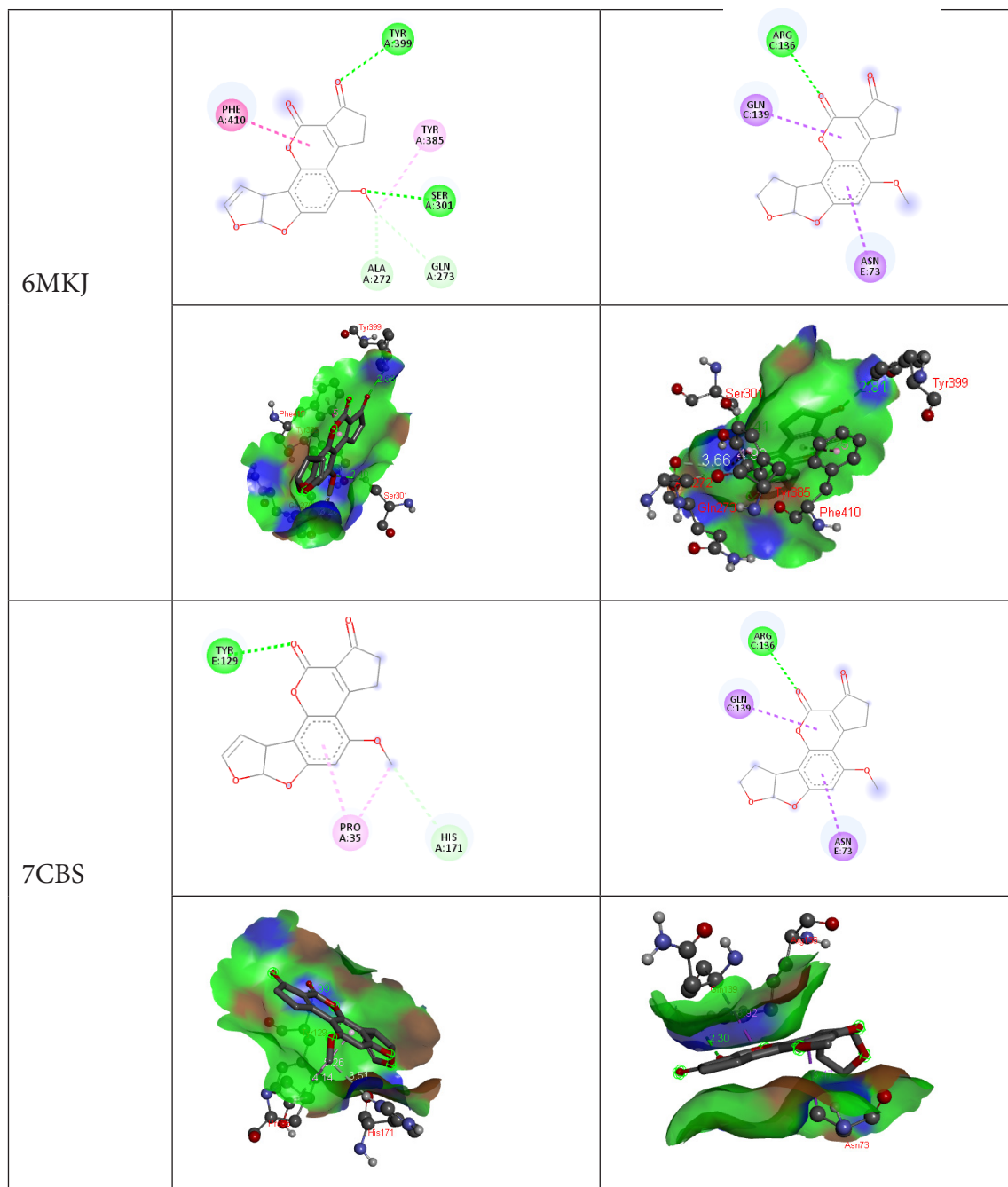
Table 4. Binding affinity of structures of the chosen probiotic cultures against aflatoxins

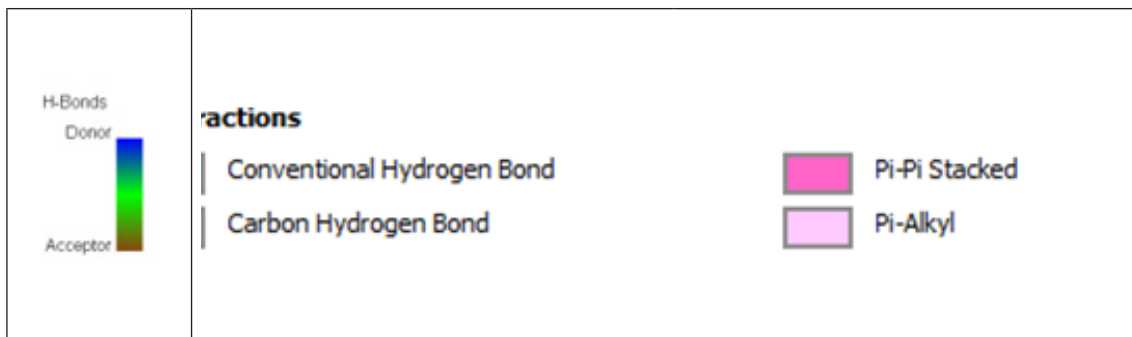
S. No.	Structure of Probiotic cultures	ΔG value			
		AFB1	AFB2	AFG1	AFG2
1	Crystal structure of SPaB basal pilin from <i>Lactobacillus rhamnosus</i> GG	-7.6± 0.32	-7.5± 0.29	-7.1± 0.07	-7.6± 0.27
2	Tertiary structure of apo-D-alanyl carrier protein of <i>Lactobacillus casei</i>	-6.9± 0.53	-6.8± 0.52	-6.3± 0.35	-6.8± 0.54
3	Distinct and essential morphogenic functions for wall and lipo-teichoic acids in <i>Bacillus subtilis</i>	-9.5± 0.55	-9.4± 0.45	-9.5± 0.30	-9.4± 0.32
4	Crystal structure of penicillin binding protein -5(PBP-5) from <i>Enterococcus faecium</i> in the closed conformation	-6.9± 0.17	-6.7± 0.19	-7.2± 0.26	-7.1± 0.23
5	Structure of the N-terminal domain of the F101 adhesin (n-flo1p) from the yeast <i>Saccharomyces cerevisiae</i>	-6.8± 0.27	-6.8± 0.26	-6.8± 0.27	-6.5± 0.17

Table 5. The 2D and 3D output structures

Probiotic culture structure	AFB1	AFB2
1HQB		
		







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