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MOLECULAR CHARACTERIZATION OF *LACTOBACILLUS* FROM BROILER AND *IN-VITRO* DETOXIFICATION OF AFLATOXIN B1

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ABSTRACT

Aflatoxin B1 (AFB1) is the most hazardous toxin in food and feed components. *Lactobacilli* are extensively studied probiotic bacteria with detoxification characteristics. This study was conducted to isolate *Lactobacillus* from broiler gut, identify them by 16S ribosomal RNA (16S r RNA) gene sequence and evaluate their detoxification ability against AFB1. A total of fourteen *Lactobacillus* isolates were obtained from poultry gut and were identified. Phylogenetic study on the base of 16S rRNA gene showed that isolates from same species and source are genetically close to each other as compared to other isolates. Out of fourteen isolates, five have shown detoxification ability against AFB1 (50 ppb), in aqueous environment at 37 °C for 6 hours. Viable cells and cell walls have removed more concentration of AFB1 as compared to cell metabolites. Isolate Cr. 4 (*L. salivarius*) removed the highest amount (92.3%) of AFB1 as compared to other isolates. It was concluded that *Lactobacillus* isolates from broiler gut are potentially active detoxifying agents against AFB1 and their detoxification ability is strain dependent.

Keywords: *Lactobacillus*, Aflatoxin B1, detoxification, *in vitro*
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INTRODUCTION

Aflatoxins (AFs) are the most dangerous mycotoxins, produced by different species of *Aspergillus* (*A. flavus, A. parasiticus* and *A. nomius*). They contaminate cereals, grains, spices and feed components during harvest, processing and storage. When these contaminated products are consumed by animal, birds or human, AFs cause health condition known as aflatoxicosis. It includes anorexia, immunosuppression, fatty liver and liver cancer. Among different types of AFs (AFB1, AFB2, AFG1, AFG2, AFM1 and AFM2), AFB1 is highly toxic. It is hepatotoxic, mutagenic, carcinogenic, teratogen and immunosuppressant (Hussain *et al.,* 2016).

Many biological and chemical agents have shown detoxification ability against AFs. Due to disadvantages of chemicals agents, biological detoxification of AFs is getting attention (Chen *et al.,* 2017). *Lactobacilli* are extensively studied probiotics from fermented products and animal gut with detoxification potential. *Lactobacilli* have shown probiotic behavior in gut by playing important role in nutrient digestion, xenobiotic metabolism and immunomodulation (Conlon and Bird, 2015). They can adsorb AFs by cell wall components (Teichoic acid), instead of covalent binding or degradation. *Lactobacilli* have shown inhibitory effect against mycelia growth of fungi (Sadiq *et al.,* 2019). They absorb AFB1 irrespective of media used for their growth (Hussain *et al.,* 2016). They removed 80-85% of the AFB1 from stored samples at 4 °C. They not only adsorbed AFB1 from medium but also prevented aflatoxicosis in mice (Liew *et al.,* 2018). *L. plantarum* (Viable and heat killed) has shown highest binding ability against AFB1, even at lower pH and high levels of bile salts (Huang *et al.,* 2017).

Lactobacillus is dominant genus in gastrointestinal tracts of broilers (Xiao *et al.,* 2017). It is documented that 10-61% gut microbial population is *Lactobacillus,* although all of them are not cultivable (Rajoka *et al.,* 2018). Different factors such as diet, medicines, antibiotics and environmental factors decide gut micro biota (Conlon and Bird, 2015). *Lactobacillus* has unique distribution among different segments of gut (Nallala and Jeevaratnam, 2015). They are identified as Gram's positive rods, Catalase negative, non-endospore forming and negative for acid fast staining. Their cell wall is tough to break. They could tolerate low pH, high phytase, protease and sensitive for lipase and amylase (Alimolaei and Golchin, 2016).

It is not possible to identify genus *Lactobacilli* up to species level by phenotypic methods. The sequence of 16S rRNA gene helps in accurate identification of all prokaryotes up to species level (Adhikari and Kwon, 2017). 16S rRNA gene sequence (1500 bp long) is a genetic marker which is along with databases is used for taxonomic characterization of microbes (Srinivasan *et al.,* 2015). 16S r RNA is part of 30 S small subunit of

ribosome and its gene is highly conserved with nine variable regions. These variable regions help in taxonomical identification of prokaryotes. The V3 region helpful for identify genus and V6 for species identification. Comparison of these sequences is even helpful in strain identification (Johnson *et al.,* 2019).

So *in vitro* detoxification ability of some broiler *Lactobacilli* isolates against AFB1 were investigated in current study. For this purpose, isolates were exposed to AFB1 in buffer and detoxification was estimated by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Isolation of *Lactobacillus* from broiler gut: *Lactobacillus* were isolated from broiler gut according to method described by Nallala and Jeevaratnam, (2015), at department of microbiology, University of veterinary and animal sciences, Lahore. For this purpose, gastrointestinal tract (GIT) of 10 broilers was obtained from local market of Lahore. Two-inch segment was cut open aseptically from each part of GIT and its contents were suspended in sterilized phosphate buffer saline (PBS, Sigma Aldrich). Gut suspension from each section was inoculated on MRS (Oxoid, UK) agar plates and incubated at 37 °C for 48 hours in order to obtain individual colonies of bacteria.

Identification of *Lactobacillus:* The isolates were subjected to Gram's staining and catalase test (Bergey and Holt, 1994) for their genus identification. Only Gram's positive and catalase negative isolates were selected for the study. Stock cultures of isolates were prepared in glycerol 15% V: V and preserved at -20 °C (Nallala and Jeevaratnam, 2015).

DNA extraction and 16S rRNA gene sequencing: DNA of each isolate was extracted by using method described by Alimolaei and Golchin, (2016) with minor modification. DNA extraction was done at Microbiology section, Quality operational laboratories, University of veterinary and animal sciences, Lahore. For this purpose, 2 mL fresh culture of each isolate was taken and centrifuged at 6000 rpm for 10 min. The pellet was freeze thawed thrice and 1mL Tris-EDTA-NaCl (TEN; pH 8) was added. It was mixed and centrifuged at 65000 rpm for 5 min. In the pellet, 1 mL Sucrose, EDTA, Tris buffer (SET Buffer; pH 8) and 50 µL lysozyme (20 mg/mL in TEN Buffer) were added. After incubation at 37 ˚C for 1 hour, 50 µL TEN buffer and 50 µL, 50 % sodium dodecyl sulphate (SDS) were added and incubated at 60˚C for 15 minutes. After cooling 50 µL, 5M sodium chloride was added and shaken to precipitate proteins. Equal volume (1:1) of phenol: chloroform was added in it and mixed gently. It was centrifuged at 10,000 rpm for 10 min at 4 ˚C. Upper clear layer was collected in a separate tube. Double volume of chilled cold ethanol was added to precipitate DNA. After washing with 70 % Ethanol, it was centrifuged at 12000 rpm, 10 min at 4 ˚C. The pellet was air dried and suspended in 25 µL TE buffer. Concentration of DNA was determined by Nano drop spectrophotometer.

Only universal forward primer 8FLP-F (GGATCCGCGGCCGCTG CAGAGTTTGATCCTGGCTCAG) was used to sequence partial 16S r RNA gene at Tsing Ke biological technologies, Beijing, China (Nawaz *et al.,* 2011). Big Dye Terminator Kit was used for sequencing reaction. Sangers Method and 96 capillary ABI3730XL System were used for sequencing.

Source and preparation of AFB1: AFB1 (Sigma Aldrich, CAS no: A6636) was purchased from local supplier. Its 10 µg/mL stock solution was prepared in PBS (Sigma Aldrich, pH: 7.1). Working solution of 50 ppb AFB1 (0.05 µg/mL) was prepared in PBS and stored in brown glass bottle at -20 °C (Drobna *et al.,* 2017).

In vitro detoxification of AFB1 by *Lactobacillus:* Activity of *Lactobacillus* isolates against AFB1 was determined by using method described by Mendoza *et al.*, (2009) with minor variations. Viable cells of all isolates were studied for detoxification ability against AFB1. All the steps were performed thrice. One mL broth culture of all isolates ($9E^{11}$ CFU/ mL) was taken. It was centrifuged at 4000 rpm for 10 minutes, bacterial cells were suspended and incubated with 50 ppb AFB1 (in PBS, pH 6.5) at 37 °C for 6 hrs. After centrifugation supernatant were dried in water bath and reconstituted in 1 mL methanol. Samples were spotted on TLC plate along with standard AFB1 and developed in chromatography tank containing mobile phase (Diethyle ether- methanol-water in V: V: V 96:3:1) and observed under UV light (Oluwafemi *et al.,* 2010).

Isolates showing detoxification ability against AFB1 were further studied to understand mechanism of detoxification. For this purpose, cell fractions (cell wall and cell cytosol) were isolated by using method described by Shrestha *et al.,* (2012). Cell fractions (cell walls and cell metabolites) were separately mixed with 1mL of AFB1 solution (50 ppb in PBS, pH 7.4, 0.1 M). They were incubated at 37 °C for different time duration (2, 4, 6 hrs). After centrifugation at 5000 rpm for 10 min, supernatants were dried in water bath (Mendoza *et al.,* 2009).

HPLC: The dried AFB1 samples were derivatized by using method described by Hussain *et al.,* (2016). HPLC system of Agilent 1100 series, C 18 Column and fluorescent detector was used to estimate concentration of AFB1. Mobile phase was acetonitrile: Methanol: Water (20:20:60; v/v/v). Excitation and emission wavelengths were 360 and 440 nm. Flow rate was 1mL/Min and

column temperature was 40 °C. Injection volume was 20 µL (Hussain *et al.,* 2016).

Statistical Analysis: Nucleotide sequences were obtained and NCBI- Basic local alignment search tool (BLAST) was performed to find out species of each bacterium. Sequences were submitted to NCBI-Gene Bank to obtain accession numbers. Neighbor joining tree was constructed by MEGA 6 software (Tamura *et al.,* 2013). Boot strap test with 500 replicates and Tamura Nei model were used for this purpose. *Lactococcus lactis* (MH549141.1) was used as an out-group (Shokryazdan *et al.,* 2014).

Detoxified of AFB1 was calculated in form of ng/mL (ppb). The data was analyzed by using one-way ANOVA of SPSS 16.0 software and Duncan's multiple range test was used to compare means. Difference between means was considered significant when $P<0.05$.

RESULTS

Isolation and initial identification of *Lactobacilli:* A total of fourteen isolates from broiler gut were identified as *Lactobacillus* (Table 1). According to macroscopic view these isolates had small to medium sized circular colonies. Their colors range from white to off white and they were transparent and greasy to dry textured on MRS agar plates. Microscopic observations showed that all of them were Gram's positive, medium to long (1.1 to 5.7 µm) rods arranged singly, in pairs or in short or long chains. They gave negative results for Catalase test. The isolates obtained were from crop (28.6%), duodenum (14.3%), jeju-ileum (21.4%), caecum (21.4%) and from colon (14.3%). The most dominant species was *L. salivarius* (50%) (Fig. 1).

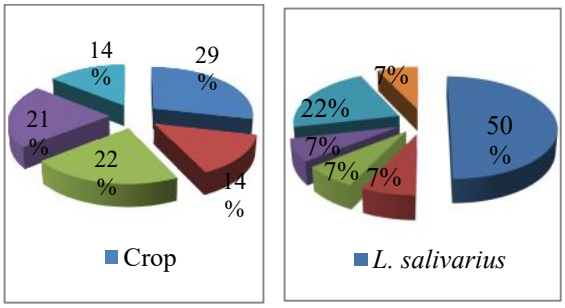

Figure 1: Percentage prevalence of *Lactobacillus*, broiler isolates with respect to: (left) different region of GIT, (right) species.

Identification of *Lactobacillus* by 16S rRNA gene: The results of the 16S rRNA gene sequencing showed that all the isolates had 97-99% similarity with 99% query coverage. 16S rRNA gene sequences of all the 14 isolates were deposited in the GenBank database under the accession numbers (MG938646- MG938659) (Table 1).

Table 1: *Lactobacillus* isolates from broiler gut, identified by using 16S rRNA gene sequences.

Sr. #	Isolates	Name of Isolate	Sequence length bp	Accession number	Nearest match species from Gene Bank (Similarity)
1	Ce.28	*L. crispatus*	1011	MG938646	EU600916.1 (99%)
2	Cr.1	*L. salivarius*	1009	MG938647	MH333261.1 (99%)
3	Cr.2	*L. salivarius*	980	MG938648	EU600916.1 (99%)
4	Cr.3	*L. salivarius*	994	MG938649	KP979479.1 (99%)
5	Cr.4	*L. salivarius*	967	MG938650	KU163336.1 (99%)
6	Co.1	*L. helveticus*	1018	MG938651	KM506757.1 (98%)
7	Co.4	*L. salivarius*	982	MG938652	MH333261.1 (99%)
8	Du.2.1	*L. salivarius*	1004	MG938653	KP979479.1 (99%)
9	Du.2.3	*L. ruteri*	945	MG938654	MF686468.1 (99%)
10	Ce.2.1	*L. agilis*	988	MG938655	MH393004.1 (99%)
11	Ce.3.1	*L. agilis*	982	MG938656	KC561112.1 (99%)
12	IL.1.1	*L. agilis*	968	MG938657	KC561112.1 (99%)
13	IL.2.1	*L. fermentum*	945	MG938658	MF582917.1 (98%)
14	IL. 2.2	*L. salivarius*	949	MG938659	KP979479.1 (99%)

Neighbor joining Phylogenetic tree: Phylogenetic tree constructed by **Neighbor joining method** (Fig. 3) showed that isolates of same species clustered together on phylogenetic tree. *L. salivarius* isolates from broiler duodenum (MG938653), ileum (MG938659), colon (MG938652) and crop (MG938647, MG938648, MG938649, MG938650) were genetically close to isolate from broiler gut (KU587802). *L. agilis* isolates from broiler caecum (MG938655, 56) were a bit at distance from isolate from broiler ileum (MG938657). *L. crispatus* isolated from broiler caecum (MG938646) was closer to isolate from poultry intestine (KU311633) as compared to isolate from poultry dropping (AB597000). *L. ruteri* from broiler duodenum (MG938654) was close to isolate

from Pig intestine (KC6145829). *L. helveticus* (MG938651) from broiler colon was close to isolate from goat milk, (KM506757). *L. fermentum* isolate from broiler ileum (MG938658) was adjacent to isolate from fodder (MF582917). Distance based phylogenetic study of 16S rRNA gene sequence showed that *Lactobacilli* from same species were genetically close to each other. According to this tree isolates from animal, birds GIT, milk and fermented products were close to each other as compared to isolates from human gut, water and sea sediments.

Detoxification ability of *Lactobacillus* against AFB1 *In-Vitro:* Results from TLC plate showed that all the *Lactobacillus* isolates from broiler gut does not have equal detoxification ability against AFB1. When incubated with 50 ppb AFB1 out of fourteen isolates, five isolates (two from crop: Cr. 3, Cr.4, three from caecum: Ce. 28, Ce. 2.1, Ce. 3.1) removed AFB1 from aqueous media at pH 6.5, after 6 hours of incubation at 37 °C temperature (Fig. 2).

According to data from HPLC, time had positive effect on detoxification of AFB1 by *Lactobacillus* isolates (Table 2).

Table 2: Detoxification of AFB1 (50ppb) by 5 *Lactobacillus* broiler isolates in aquous medium. Effect of fractions (viable cells: VC, cell walls: CW, cell metabolites: CM) and time of incubation (hrs.) on detoxification of AFB1.

Isolate	Viable Cells			Cell metabolites			Cell Walls		
	2hrs	4 hrs	6 hrs	2hrs	4 hrs	6 hrs	2hrs	4 hrs	6 hrs
***L. crispatus* (CE.28)**	45.19 ± 0.15 [b]	47.07 ± 0.01 [b]	48.64 ± 0.27 [c]	42.55 ± 0.01 [a]	42.49 ± 0.14 [a]	43.64 ± 0.06 [a]	45.74 ± 0.005 [a]	45.74 ± 0.01 [b]	46.36 ± 0.02 [b]
***L. agilis* (CE. 2.1)**	43.33 ± 0.1 [c]	43.54 ± 0.36 [c]	46.17 ± 0.01 [d]	31.98 ± 0.001 [e]	32.4 ± 0.002 [e]	33.39 ± 0.004 [e]	42.74 ± 0.01 [b]	43.7 ± 0.04 [c]	45.82 ± 0.006 [c]
***L. agilis* (CE. 3.1)**	41.35 ± 0.03 [d]	41.54 ± 0.01 [d]	45.70 ± 0.06 [e]	37.39 ± 0.06 [b]	37.47 ± 0.21 [b]	37.73 ± 0.01 [b]	41.72 ± 0.006 [d]	41.84 ± 0.04 [d]	45.74 ± 0.001 [d]
***L. salivarius* (CR. 3)**	38.56 ± 0.17 [e]	39.52 ± 0.01 [e]	49.96 ± 0.01 [b]	32.63 ± 0.005 [c]	32.68 ± 0.008 [d]	34.05 ± 0.1 [d]	38.45 ± 0.01 [e]	39.54 ± 0.01 [e]	45.63 ± 0.001 [e]
***L. salivarius* (CR. 4)**	46.53 ± 0.001 [a]	49.91 ± 0.021 [a]	49.98 ± 0.06 [a]	32.25 ± 0.02 [d]	34.02 ±0.17 [c]	34.79 ± 0.2 [c]	42.24 ± 0.03 [c]	49.88 ± 0.09 [a]	49.96 ± 0.01 [a]

The Duncan's test showed that mean difference between detoxified AFB1 by *Lactobacillus* isolates is significant if P< 0.05. Superscript "a" indicates highest amount of AFB1 detoxified by isolates, followed by "b, c, d, e".

As cell fractions are concerned, viable cells removed more amount of AFB1 as compared to cell walls. Minimum detoxification was shown by cell metabolites. Viable cells and cell walls of Cr. 4 removed maximum amount of AFB1 (99.96 %, 99.92 %) as compared to other isolates. Viable cells of isolate Ce.28 removed 97.2%, Ce.2.1: 92.3%, Ce.3.1: 91.4% and Cr.3: 99.92 % AFB1 from media.

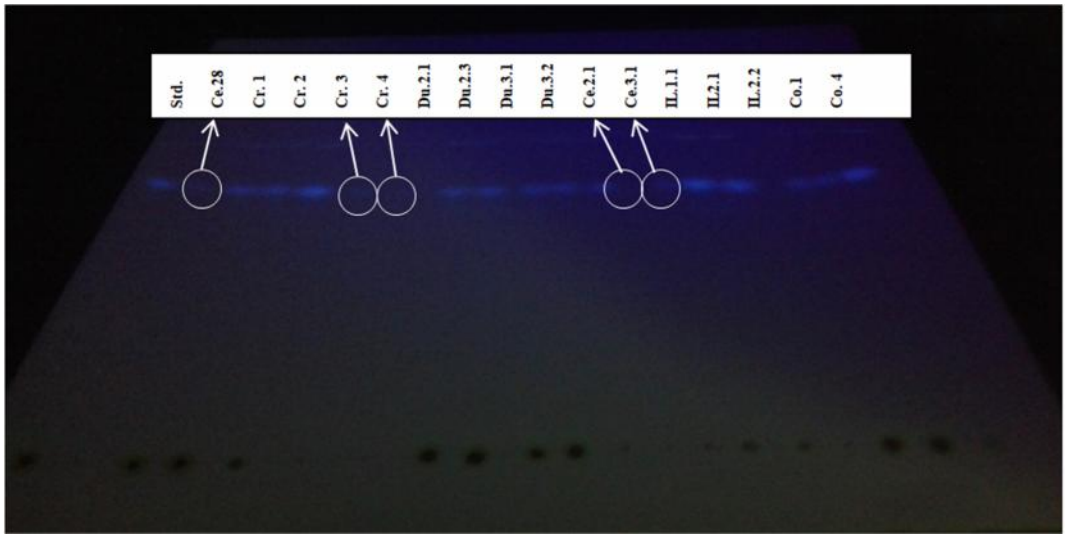

Figure 2: Detoxification ability of fourteen *Lactobacillus,* broiler isolates against AFB1(50 ppb) on TLC plate. Thin layer chromatography (TLC), Crop (Cr.) Duodenum (Du.), Ileum (IL.), Caecum (Ce.) and Colon (Co.).

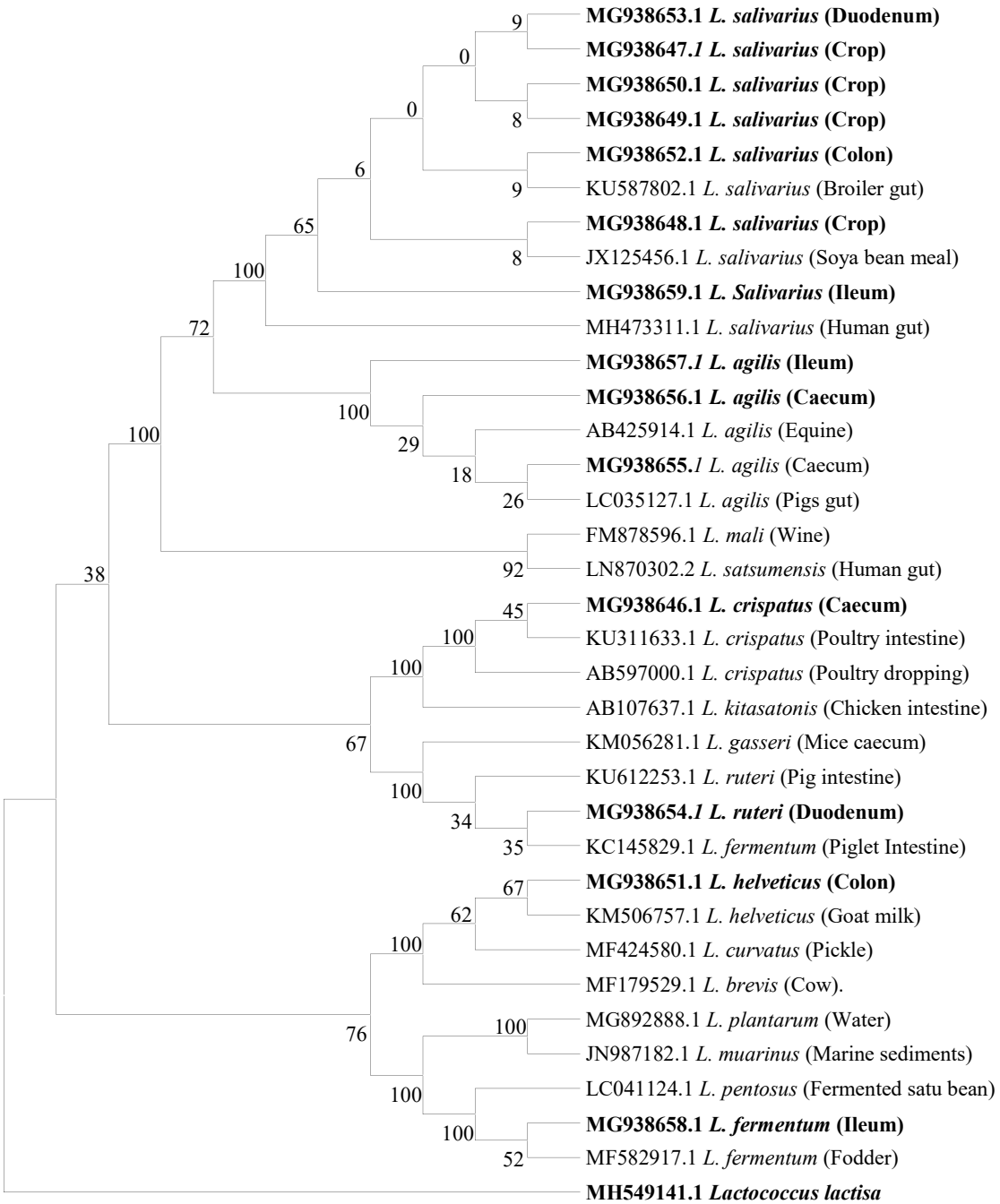

Figure.3: Phylogenetic tree based on 16S rRNA gene sequence analysis, using the Neighbor-Joining method (Saitou and Nei, 1987). The out group was *Lactococcus lactis* (MH549141). Fourteen out of 34 nucleotide sequences were from this study and remaining twenty sequences belong to *Lactobacillus* species obtained from the Gene Bank (NCBI). Boot strap values up to 100 % are indicated at the nodes of the tree.

DISCUSSION

Previous studies have shown that animal gut is best choice for isolation of bacteria with highest detoxification ability against mycotoxins. As detoxification ability of bacteria increases in presence of bile salts (Mendoza *et al.*, 2009). It is documented that 10-61% gut microbial population is *Lactobacillus,* although all of them are not cultivable (Rajoka *et al.,* 2018). It has been reported that the relative abundance of *Lactobacillus* varies among different segments of the GIT in chickens (Ranjitkar *et al.,* 2016). Analysis of chicken

GIT revealed that *Lactobacillus* is dominant genus in upper GIT, whereas *L. salivarius* and *L. aviarius* are dominant species of this genus (Adhikari and Kwon, 2017). Studies have shown that poultry crop and caecum have high population of *L. salivarius* (36% and 28%) (Adhikari and Kwon, 2017). *Lactobacillus* species are dominant in crop than in caecum (Ranjitkar *et al.* 2016). Findings of Wang *et al.* (2014) supported this study by conclusion that *Lactobacillus* has highest diversity in crop as compared to Caecum. One of the most common methods to identify novel bacterial isolates is phylogenetic analysis of 16s rRNA gene sequences (Chen *et al.,* 2017). Isolates with 97-99% gene sequence similarity, can be placed in same species (Choi and Lee, 2015).

Previous studies have shown that *Lactobacilli* are probiotics which improve intestinal health of host (Darsanaki *et al.,* 2014). *Lactobacilli* also play role in reducing level of AFB1. Live cells have shown highest binding efficiency (98%) against AFB1 (Liew *et al.,* 2018). Stability of the complexes formed depends on strain, treatment, and environmental conditions (Haskard *et al.,* 2001). Five different species of *Lactobacillus* (*L. acidophilus, L. brevis, L. casei, L delbruekii* and *L. plantarum*) decrease level of AFB1 about 44.5% (5ng/g) in maize grains (Oluwafemi *et al.,* 2010). This difference in binding AFs could be due to difference in structure of cell wall teichoic acid and peptidoglycan which are responsible for this detoxification (Haskard *et al.,* 2001). Intracellular metabolites (Enzymes) also play role in detoxification of AFB1. *L. casei* was considered to degrade AFB1 by different enzymes *in vivo* (Zuo *et al.,* 2013). *L. fermentum* metabolites reduced AF level from 88.8% to 99.8% (Ghazvini *et al.,* 2016). According to previous work lactic acid bacteria has ability to bind toxins such as AFB1 from liquid media. *L. rhamnosus* adsorb about 80% of AFB1 within an hour (Haskard *et al.,* 2001). After 4 hours of incubation, *Lactobacillus* removed 30 % of AFB1. Time has positive relation with detoxification of toxins. More is the time of incubation, more will be the detoxification (Mendoza *et al.,* 2009). Lactic acid bacteria were safe for bio-detoxification of AF, so their use should be encouraged (Oluwafemi *et al.,* 2010).

Conclusions: There is diversity in distribution of *Lactobacillus* in broiler gut. That's why different cultivable *Lactobacilli* are found in different regions of broiler gastro intestinal tract. Some isolates are specifically present in a region. Neighbor joining phylogenetic tree of *Lactobacillus* isolates showed that isolates from poultry gastro intestinal tract were genetically closer to isolates from gut of other animal, milk and fermented product than those isolated from human gut, water and sea sediments. Detoxification of AFB1 by *Lactobacillus* was strain dependent. Isolates from crop and caecum of broiler are more active against AFB1 as compared to isolates from other regions of gut. Cell walls of isolates played significant role in detoxification of AFB1 as compared to cell metabolites. Amount of AFB1 removed increases by increasing time of incubation.

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Author's contribution: Rubina Yasmeen (RY) and Abu Saeed Hashmi (ASH) designed, conducted the study, processed samples and collected the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

REFERENCES

Adhikari, B. and Y.M. Kwon (2017). Characterization of the culturable subpopulations of *Lactobacillus* in the chicken intestinal tract as a resource for probiotic development. Front. Microbiol. 8: 1389-1397 doi: 10.3389/fmicb.2017.01389

Alimolaei, M. and M. Golchin (2016). An efficient DNA extraction method for *Lactobacillus casei*, a difficult to lyse bacterium. Int. J. Enteric. Patho. 4(1): e32472 doi: 10.17795/ijep32472.

Bergey, D.H. and J.G. Holt (1994). Bergey's Manual of Determinative Bacteriology. 9th Edition. Lippincott Williams & Wilkins. 175-184 p. Baltimore, US

Chen, F.L. Zhu and H. Qiu (2017). Isolation and probiotic potential of *Lactobacillus salivarius* and *Pediococcus pentosaceus* in specific pathogen free chickens. Brazilian J. Poultry Sci . 9(2): 325-332

Choi, W.Y. and H.Y. Lee (2015). Complete nucleotide sequence of the 16S rRNA from *Lactobacillus paracasei* HS-05 isolated from women's hands. AMB Express, 5(1): 78-83. https://doi.org/ 10.1186/s13568-015-0158-8

Conlon, M.A. and A.R. Bird (2015). The impact of diet and lifestyle on gut microbiota and human health. Nutrients 7(1): 17–44. doi: 10.3390/nu7010017

Darsanaki, R.K., M.H. Kolavani, M.M.D. Chakoosari, S.E. Shalkeh and A. Tajehmiri (2014). Biological control of aflatoxin b1 by probiotic bacteria. DAMA Int. 3(1): 2319–5037.

Drobna, A., D. Rauova, H. Majekova, G. Greif and P. Mikus (2017). Antifungal activity and aflatoxin binding ability of *Lactobacillus* species isolated from lamb and goatling stomach mucus. J. Food and Nutrition Res. 56 (3): 255–264

Ghazvini, R.D., E. Kouhsari, E. Zibafar, S.J. Hashemi, A. Amini and F. Niknejad (2016). Antifungal activity and Aflatoxin degradation of *Bifidobacterium bifidum* and *Lactobacillus fermentum* against toxigenic *Aspergillus parasiticus*. The Open Microbiol. J. 10: 197-201

Haskard, C.A., H.S. El Nizami, P. Kannkaanpaa, S. Salminen and J.T. Ahokas (2001). Surface binding of AFB1 by Lactic acid bacteria. Appl. Environ. Microbiol. 67(7): 3086-3091. doi: 10.1128/AEM.67.7.3086-3091.2001

Huang, L., C. Duan, Y. Zhao, L. Gao, C. Niu, J. Xu and S. Li (2017). Reduction of Aflatoxin B1 toxicity by *Lactobacillus plantarum* C88: A potential probiotic strain isolated from Chinese traditional fermented food "Tofu". PLoS ONE. 12(1): e0170109.doi:10.1371/journal. pone.0170109

Hussain, Z., H. Rehman, S. Manzoor, S. Tahir and M. Mukhtar (2016). Determination of liver aflatoxicosis in broiler chickens. Vet. Clinical Pathol. 45(2): 330-334. https://doi.org/10.1111/vcp.12336

Johnson, J.S., D.J. Spakowicz, B.Y. Hong, L.M. Petersen, P. Demkowicz, L. Chen, S.R. Leopold, B.M. Hanson, H.O. Agresta, M. Gerstein, E. Sodergren and G.M. Weinstock (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nat. Commun. 10**:** 5029-5040

Liew, W.W.P., Z.N. Adilah, L.T.L. Than and S.M. Redzwan (2018). The binding efficiency and interaction of *Lactobacillus casei* Shirota toward Aflatoxin B1. Front. Microbiol. 9: 1503-1515. doi: 10.3389/fmicb.2018.01503

Mendoza, A., H.S. Garcia and J.L. Steele (2009). Screening of *Lactobacillus casei* strains for their ability to bind aflatoxin B1. Food Chem. Toxicol. 47:1064–1068

Nallala, V. and K. Jeevaratnam (2015). Molecular characterization of bacteriocinogenic, antifungal and probiotic lactic acid bacteria isolated from chicken gastrointestinal tract. Adv. Microbiol. 5: 644-660. http://dx.doi.org/10.4236/aim.2015.59067

Nawaz, M., J. Wang, A. Zhou, C. Ma, X. Wu, J.E. Moore, B.C. Millar and J. Xu (2011). Characterization and transfer of antibiotic resistance in Lactic acid bacteria from fermented food products. Curr. Microbiol. 62:1081–1089 DOI 10.1007/s00284-010-9856-2

Oluwafemi, F., M. Kumar, R. Bandyopadhyay, T. Ogunbanwo and K.B. Ayanwande (2010). Bio-detoxification of aflatoxin B1 in artificially contaminated maize grains using lactic acid bacteria. Toxin review 29(3–4): 115–122.

Rajoka, M.S.R., H.F. Hayat, S. Sarwar, H.M. Mehwish, F. Ahmad, N. Hussain, S.Z.H. Shah, M. Khurshid, M. Siddiqu and J. Shi (2018). Isolation and evaluation of probiotic potential of lactic acid bacteria isolated from poultry intestine. Microbiol. 87(1): 116–126.

Ranjitkar, S., B. Lawley, G. Tannock and R.M. Engberg (2016). Bacterial succession in the broiler gastrointestinal tract. Appl. Environ. Microbiol. 82 (8): 2399–2410. doi: 10.1128/AEM.02549-15

Sadiq, F.A., B. Yan, F. Tian, J. Zhao, H. Zhang and W. Chen (2019). Lactic Acid Bacteria as Antifungal and Anti-Mycotoxigenic Agents: A Comprehensive Review. Compr. Rev. Food Sci. Food Saf. 18(5): 1403-1436

Shrestha, B., K.I. Ansari, A. Bhan, S. Kasiri, I. Hussain and S.S. Mandal (2012). Homeodomain-containing protein HOXB9 regulates expression of growth and angiogenic factors, facilitates tumor growth *in vitro* and is overexpressed in breast cancer tissue. FEBS J. 279: 3715-3726.

Shokryazdan, P., R. Kalavathy, C.C Sieo, N.B. Alitheen, J.B. Liang, M.F. Jahromi and Y.W. Ho (2014). Isolation and characterization of *Lactobacillus* strains as potential probiotics for chickens. Pertanika J. Trop. Agric. Sci.37 (1): 141 - 157 ISSN: 1511-3701

Srinivasan, R., U. Karaoz, M. Volegova, J. Mac Kichan, M. Kato-Maeda and S. Miller (2015). Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. PLoS ONE 10 (2): e0117617.doi:10.1371/journal. pone.0117617

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Bio. Evo. 30 (12): 2725–2729

Wang, L., M. Fang, Y. Hu, Y. Yang, M. Yang and Y. Chen (2014). Characterization of the most abundant *Lactobacillus* species in chicken gastrointestinal tract and potential use as probiotics for genetic engineering. Acta Biochem. Biophys. Sin. 46 (7): 612-619. doi: 10.1093/abbs/gmu037.

Xiao, Y., Y. Xiang, W. Zhou, J. Chen, K. Li and H. Yang (2017). Microbial community mapping in intestinal tract of broiler chicken. Poultry Sci. 96 (5): 1387–1393 http://dx.doi.org/10.3382/ps/pew372

Zuo, R., J. Chang, Q. Yin, P. Wanga, Y. Yang, X. Wang, G. Wang and Q. Zheng (2013). Effect of the combined probiotics with aflatoxin B1-degrading enzyme on aflatoxin detoxification, broiler production performance and hepatic enzyme gene expression. Food Chem. Toxicol. 59: 470–475.