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Detoxification of Aflatoxin B1 by Isolating and Screening *Bacillus* species from the Gastrointestinal Tract of Broilers

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Abstract

Aflatoxins, which are widely detected in cereals, are capable of causing diseases in humans and animals. The aim of this study was to isolate and screen *Bacillus* species. as direct-fed microbials to the degrading of Aflatoxin B_1 (AFB_1) in an in vitro condition. Ten hundred *Bacillus* isolates were obtained from broilers' gastrointestinal tracts. Probiotic characteristics such as antibacterial activity, antibiotic susceptibility, acid and bile tolerance, aggregation and coaggregation assays, cell surface hydrophobicity, biofilm formation and extracellular enzyme production were evaluated. The reduction of AFB_1 concentration was carried out with high-performance liquid chromatography (HPLC). Residual AFB_1 toxin in the final product was detected. The morphological and biological assayed followed by analysis of 16S rRNA gene sequence were carried out for identification of *Bacillus* species. Out of 100 *bacillus* species, six isolates, including MA57, MA58, MA71, MA73, MA81 and MA82 gamma hemolysis. About two *Bacillus* strains exhibited maximum antimicrobial activity. Isolates showed a good tolerance to acid and bile salt conditions. The aggregation and coaggregation activity of MA82 was higher than MA71. Both isolates were able to strongly biofilm formation. Extracellular enzyme production of the two tested *Bacillus* species was various. The MA82 was more effective in biodegrading AFB_1 (up to 75%). Analyzing the 16S rRNA gene sequence showed that it belonged to the strain of *Bacillus* species MBIA2.40 (92.98% Identification). These results suggest that *Bacillus sp.* MBIA2.40 should not only be used as probiotics but also may be as adsorptive for aflatoxin B_1.

Introduction

Aflatoxins are considered the most important secondary toxic metabolites of mycotoxins, which are naturally synthesis by *Aspergillus flavus*, *Aspergillus nomius* and *Aspergillus parasiticus* (Ting *et al*., 2020; Xu *et al*., 2020). It is discovered that these secondary metabolites are mainly found in cereals and can result in carcinogenic outcomes in humans and animals (Emmanuel *et al*., 2020). The International Agency for Research on Cancer recorded aflatoxin B_1 as a group 1 carcinogenic substance (Alshannaq and Yu, 2017; Haque *et al*., 2020). It was reported that *Bacillus* species introduced as the direct-fed microbial (DFM) (Paneru *et al*., 2023) and AFB_1 adsorption (Huang *et al*., 2024) in human and animal health. More evidence showed that some species of *Bacillus* genes such as *Bacillus subtilis* (Huang *et al*., 2024), *Bacillus licheniformis* (Rao *et al*., 2017), *Bacillus Albus* YUN5 (Kumar *et al*., 2023) and *Bacillus Megaterium* (Cheng *et al*., 2023) were effective in reduction of aflatoxin. Therefore, one of the practical ways to reduce aflatoxin B_1 is by applying microorganisms, as demonstrated by previous research. The technical literature involves microorganisms, including lactic acid bacteria, saccharomyces cerevisiae, bacillus genes and others, such as polysaccharides and bacterial cell wall peptidoglycans can degrade mycotoxins in various ways (Chlebicz and Salizewska, 2019).

Rosario *et al*. (2015) showed that three DFM candidates, including *Bacillus amyloliquefaciens*, *Bacillus megaterium* and *Bacillus subtilis* were able

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to degrade AFB_1 in in-vitro condition. *Bacillus subtilis* ASN0B60 was isolated from the fish gut as a probiotic and it was reduced by 81.5, 60 and 80%for AFB_1, AFM_1 and AFG_1, respectively (Gao *et al*., 2011). It was documented that after 72 h *Pseudomonas aeruginosa* N17-1 on nutrient broth medium could be able to degrade 82.8, 46.8 and 31.9 % for AFB_1, AFB_2 and AFM_1, respectively, (Sangare *et al*., 2014). All these data show that there is a great demand for a new, practical way to reduce or inactivate the harmful effects of AFB_1.

Thus, this study was conducted to isolate and discover aerobic spores from the digestive tract of broiler chickens, which are capable of bio-transforming AFB_1. Isolates were examined for probiotic characteristics and diminished AFB_1 in in vivo conditions. Finally, these features could be suitable for competitive elimination or probiotic agents, as well as AFB_1 reduction.

Materials and Methods

Preparation of samples, bacterial isolation and growth conditions

Experiments were conducted in accordance with guidelines for the care and use of animals in agricultural research and teaching at Bu-Ali Sina University, Hamedan, Iran, for one year. Fifty healthy of ross broilers and native chickens at 42 days that received any medical treatment or antibiotics were selected. Fresh samples of fecal from the floor of a house and the contents of the digestive tract (crop, jejunum, ileum, and cecum) were collected under sterile conditions. Initially, the samples were diluted 1:1 (wt/vol) in the buffered peptone water and homogenized. Then, aerobic spore-forming isolates were selected by heat (Koransky *et al*., 1978). The homogenized samples were further diluted in the 1:10 peptone water. Samples were incubated at 80°C for 15 min and cultured on the nutrient agar medium. After 24 or 48-hour incubation at 37 °C, colonized samples that showed different morphology were selected and were purified by plated on nutrient agar medium. A total of 100 colonies were purified and cultured on the blood agar medium containing 5% defibrinated sheep blood and incubated for 24 h at 37°C to detect their hemolytic activity. Gama hemolytic isolates were selected and these isolates were treated in some Difco heart-infusion broth (HIB) with 30% glycerol and stored at -70°C.

Antibacterial activity assay

The antibacterial activity of the isolates against some poultry pathogens (such as *Salmonella* serotypes *typhimurium* and *Enteritidis*, *Ecoli* serotypes O1:K1, O2:K1 and O78:K80) was determined by the agar well diffusion method (Jin *et al*., 1996).

Antibiotic susceptibility test

The antibiotic sensitivity test was determined using commercial discs (Padtan Teb, Iran). Bacillus isolates were incubated for 24 h at 37°C on the Muller Hinton agar plate. The diameters of the clear zone that appeared around the discs were measured and recorded using a digital calliper (Treagan and Pulliam, 1982).

Acid tolerance

A modified method conducted by Mayra-Makinen *et al*. (1983), Conway *et al*. (1987) and Jin *et al*. (1998) was applied to this test. At first, new cultures of strains in sterile tubes were washed twice with phosphate-buffered saline (PBS). Then, a 0.50 mL solution was transferred to 10 mL of the sterile PBS. Various pH solutions such as 2, 3, 4 and 5 (adjusted using 8 M HCl) were added to each tube. All tubes were incubated at 37°C for 0, 1, 2.30 and 4 hours and the viable cell was counted on the nutrient agar.

Bile salt tolerance

The bile tolerance of the candidates was evaluated by the method developed by Gilliland *et al*. (1984). Briefly, new cultures of strains in sterile tubes were washed twice with phosphate-buffered saline (PBS). Then, 0.25 mL of the bacterial suspension was inoculated into the 5 mL nutrient broth and 0.3% oxgall was added into all. Each tube was incubated at 37°C for 0, 1, 2:30 and 4 hours and the viable cell was counted on the nutrient agar.

Cell surface hydrophobicity

Bacterial adhesion to hydrocarbons test was performed according to Gusils *et al*. (1999). The fresh cultures of bacteria (12 to 18 h) were removed at the early of the growth phase, then they were three times washed with PBS to an OD600 (optical density at 600 nm) of 0.5 to 0.7. The amount of 3 mL of washed cells was poured off to the test tubes containing different volumes of test hydrocarbon (n-hexadecane, toluene, and xylene). The percentage hydrophobicity was calculated by following the equation:

$$\%\ \text{hydrophobicity} = \frac{(OD_{600}\ \text{before mixing} - OD_{600}\ \text{after mixing})}{OD_{600}\ \text{before mixing}} \times 100,$$

Where OD600 is the optical density at 600 nm.

Aggregation and coaggregation assays

Aggregation and coaggregation assays were carried out according to Collado *et al*. (2008) and Tuo *et al*. (2013), with some modifications. The collected overnight cell cultures were diluted with PBS. The OD_{600} absorbance was adjusted to 0.25 ± 0.5 to achieve 10^7-10^8 CFU/mL bacteria number standardize. A 500 µl of bacterial and pathogens

suspensions were mixed, vortexed and then incubated at 20°C and 37°C for different times (0, 2, 16, 20, 24 h). Aggregation percentage was calculated as follows: $[1-At/Ao]\times100$

Where, A_t show the absorbance at time t = 5 h and A_o the absorbance at time t = 0 h. For coaggregation test, 2 mL of each bacterial suspension and pathogen suspension were mixed and incubated at 37°C without emotion. The coaggregation percentage was calculated as follows:

$$\left[1-\frac{Amix}{\frac{Asalm+Aprobio}{2}}\right]\times100$$

, where A_{probio} and A_{salm} represent A_{600nm} of the separate bacterial suspensions in control tubes and A_{mix} represents the absorbance of the mixed bacterial suspension at 5 h

Biofilm formation

The microtiter plate test for finding biofilm formation was conducted by Dosler and Karaaslan (2014). Briefly, the fresh cultures of cells were transferred to the sterile tubes containing 5 mL of tryptic soy broth. Then, the test tubes were incubated at 37°C with agitation (180 rpm) for 24 h. Second step, 100 µL of bacterial suspension (1×10^7 CFU/mL) was added to the microplate (8 replicates), and incubated at 37°C for 24 h. In step three, the microplate was three washed with PBS and dried in air. In step four, the process of coloring and fixing was completed. Tryptic soy broth without bacteria was used as the negative control. *Peudomonas aeruginosa* (ATCC 25873) was used as a biofilm producer control strain.

Extracellular enzymes production

Protease activity

For protease activity, a single colony of overnight culture of bacteria was inoculated on skim milk agar plates and incubated 24 hours at 30°C for 24 h. A zone of clearance around colony indicated the production of protease (Mosca *et al*., 2003).

Amylase activity

A single colony of overnight culture of bacteria was plated on media starch agar and incubated at 37°C for 24 hours. After overnight incubation, the plates were immersed with 1% Lugol iodine solution. Amylase activity was indicated by zone clear around the colony (Sumathi *et al*., 2011).

Lipase activity

For lipase activity, the spirit blue culture medium was supplemented with olive oil emulsion according to the manufacturer's instructions. Then, cell cultures were inoculated with spirit blue medium. The plates were inverted and incubated for 24 hours. Lipase activity was determined by the appearance of a clear zone (oil and water) in the presence of a light source (Azirah *et al*., 2016).

Aflatoxin B_1 binding assay

The overnight of bacteria cultures were adjusted to the 7th McFarland tube, centrifuged (3,000 × g, 15 min) and washed twice with PBS. Aflatoxin solution (concentration of 2 mg/l) was prepared by dissolving powder of aflatoxin B_1 (Sigma-Aldrich, Steinheim, Germany) and a mixture of benzene/acetonitrile (97:3 vol/vol). The working solution of AFB_1 (5µg/mL) was prepared in PBS. After, cell cultures were dissolved in 1.5 mL of PBS (5µg/mL AFB_1) incubated at 37°C for four h. Then, cell cultures were centrifuged (3,000 × g, 15 min) and supernatant was collected for AFB_1 quantification (Peltonen *et al*., 2000). HPLC estimated the AFB1 concentration.

Identifying the strain through the 16S rRNA sequencing method

DNA extraction and PCR amplification

The extraction of genomic DNA was done according to the boiling method. Amplification of the 16s rRNA gene was performed using the universal primers.

Forward primer: 5-CAACAGAGTTTGA TCCTGGCTCAG-3. Reverse Primer: 5-GCTTAAGGAGGTGATCCAGCC-3. In order to determine 16s rRNA gene segment sequencing, the sample was sent to Macrogen Company of South Korea, where they were sequenced utilizing an Automatic DNA Sequencer 3730XL device.

Statistical analysis

The results were analyzed for statistical significance ($P < 0.05$) using the GLM procedure of SAS/STAT® 9.4 (SAS Institute, 2013). Also, Duncan's multiple range tests were used for the comparison of means ($P < 0.05$).

Results

Antibacterial activity

The six strains were experimented using the well diffusion assay to assess the production of the antimicrobial compounds by these isolates towards some gram-negative and gram-positive pathogens (Table 1). There was considerable variability in their ability to inhibit the growth of pathogens. The MA71 and MA82, which were able to produce antimicrobials effective against both *Ecoli* serotypes and *Salmonella* serotypes in an in vitro condition, were used for further testing.

Table 1. The antagonistic activity demonstrated by the six selected Bacillus strains assayed by measuring the zone diameter around the wells

Strains	Inhibitory zone (mm)				
	Escherichia coli			*Salmonella*	
	O1:K1	O2:K1	O78:K80	*Thyphimorium*	*enteritidis*
MA57	8.47^{c}	8.67^{c}	8.00^{c}	10.53^{c}	10.77^{c}
MA58	2.53^{e}	3.17^{e}	2.63^{e}	3.83^{d}	2.77^{e}
MA71	10.07^{b}	10.57^{b}	10.63^{b}	12.23^{a}	11.97^{a}
MA73	1.37^{f}	1.07^{f}	0.97^{f}	3.77^{d}	4.03^{d}
MA81	6.13^{d}	6.27^{d}	6.00^{d}	12.00^{b}	11.70^{b}
MA82	13.13^{a}	13^{a}	13.03^{a}	12.37^{a}	11.97^{a}
SEM	0.033	0.030	0.043	0.06	0.045
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means with common superscripts in same column are not significantly different ($P < 0.05$). *SEM*: standard error of the means.

Antibiotic sensitivity

Antibiotic susceptibility profiles of the selected *Bacillus.* spp are shown in Table 2. Both DFM candidates were sensitive to gentamycin, ciprofloxacin, ampicillin and penicillin. The MA82 showed resistance to erythromycin and tetracycline.

Acid and bile salt tolerance

The survival rate of the isolates in different pH levels (2, 3, 4 and 5) and bile salt tolerance (0, 0.3% ox bile) for 0, 1, 2:30 and 4 h incubation are shown in figures 1 and 2. During 4-hour incubation, the survival rates (log $_{10}$ CFU/mL) of both candidates showed similar trends in pH 2 and 3 ($P > 0.05$). The survival rate of MA82 was significantly higher than MA71 in pH 4 and 5 at different times (5.3-6.89 log $_{10}$ CFU/mL for MA71 and 6.3-7.72 log $_{10}$ CFU/mL for MA82). The MA82 showed higher resistance to bile salts rather than MA71 in pH 4 and 5after 4 and 1 h incubation time, respectively (5.96 and 5.27 log $_{10}$ CFU/mL versus 0 and 5.3 log $_{10}$ CFU/mL) ($P < 0.05$). Candidate MA82 were more resistant rather than MA71 to bile salts in pH 6 and 7 at different times ($P < 0.05$).

Table 2. Susceptibility of candidates to various antibiotics.

Antibiotics	Strains	
	MA71	MA82
Gentamicin	*S*	*S*
Chloramphenicol	*S*	*S*
Ciprofloxacin	*S*	*S*
Ampicillin	*S*	*S*
Erythromycin	*R*	*R*
Tetracycline	*S*	*R*
Penicillin	*S*	*S*

R = Resistant; S = Susceptible.

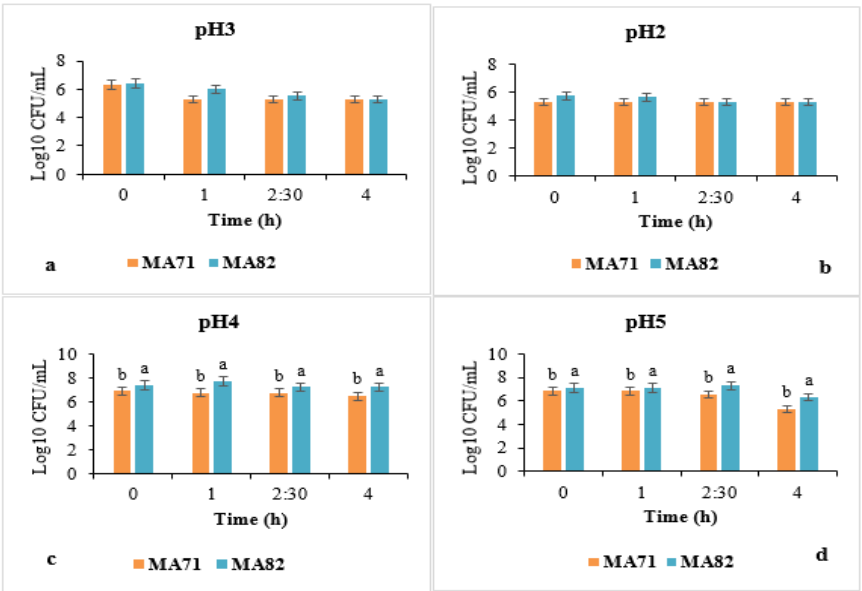

Figure 1. Number of *Bacillus.* spp bacteria (log CFU/mL) after incubation for 0, 1, 2:30 and 4 h in nutrient agar at various pH. (Means ± SD)

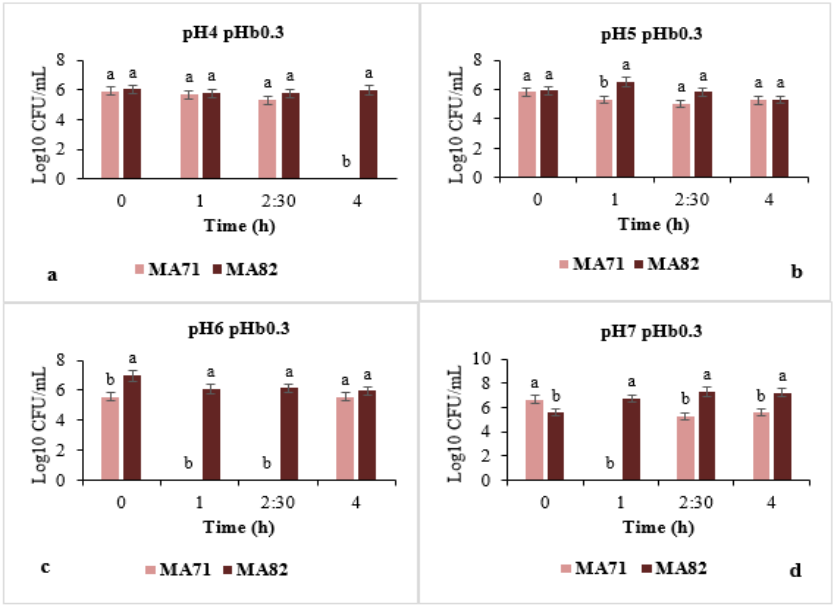

Figure 2. Number of *Bacillus*. spp bacteria (log cfu/mL) after incubation for 0, 1, 2:30 and 4 h in 0.3 % bile salts in nutrient agar at various pH. (Means ± SD)

Cell surface hydrophobicity

The adhesion percentages of strains to toluene, xylene and n-hexadecane are shown in Table 3. The MA82 has exhibited higher adhesion than the MA71. The hydrophobicity for MA82 to toluene, n-hexadecane and xylene was 67.67, 67.67, and 55.67% respectively.

Aggregation and coaggregation activity

Two bacillus strains tested showed aggregation of 26.16% and 25.82%, respectively, for isolates MA82 and MA71 after 5 h incubation at 37ºC (Table 4). No significant differences were found for coaggregation among strains with *Escherichia coli* serotypes and *Salmonella typhimurium*.

Table 3. Adhesion to hydrocarbons of strains as measured using the BATH test (%).

Strains	Tuloen	Xylen	N-hexadecan
MA71	55.00^{b}	49.67	55.00^{b}
MA82	67.67^{a}	55.67	67.67^{a}
SEM	2.357	1.856	1.105
P-value	0.021	0.084	0.001

SEM: Standard error of the means; Means with common superscripts in the same column are not significantly different ($P < 0.05$).

Table 4. Aggregation and coaggregation percentages of strains after 5 h incubation.

Strains	*Escherichia coli* (%)			*Salmonella* (%)		*Staphylococcus*	Aggregation
	O1:K1	O2:K1	O78:K80	*typhimurium*	*enteritidis*	*oreus* (%)	(%)
MA71	19.76	20.02	19.95	18.65	17.38^{b}	17.53^{b}	25.82
MA82	19.36	20.00	24.22	20.45	19.04^{a}	20.57^{a}	26.16
SEM	0.219	0.064	0.777	0.355	0.119	0.198	1.186
P-value	0.321	0.807	0.060	0.069	0.010	0.008	0.856

SEM: Standard error of the means; Means with common superscripts in the same column are not significantly different ($P < 0.05$).

Biofilm formation

Biofilm formations of isolates are shown in Table 5. It is suggested that biofilms could adhere on solid surfaces or substrates. In this study, the two isolates showed strong biofilm.

Extracellular enzymes production

The results of extracellular enzyme production by two isolates are shown in Table 6. The zone inhibition (total diameter minus the diameter of the colony) was considered proportional to the enzymatic activity. Two bacillus isolates exhibit moderate protease activity (7 and 8 mm for isolates MA71 and MA82, respectively) that were observed around the colony. For lipase assay, both isolates showed high intensity (9 and 10 mm) of extracellular enzyme production. Amylase activity of the MA82 was very high around the colony, while isolate MA71 exhibited amylase activity with an inhibition zone (9 mm).

Table 5. The biofilm formation of strains.

Strains		Negative control[1]	Positive control[2]
MA71	MA82		
+	+	-	+

[1] Medium without bacteria. [2] *Pseudomonas aeruginosa* (ATCC25873); -: No biofilm; +: strong biofilm

Table 6. Intensity of extracellular enzyme production.

Strain	amylase	protease	lipase
MA71	+++	++	+++
MA82	++++	++	+++
Negative control	-	-	-

-, nil (no halo); +, low (1-4 mm halo); ++, (5-8 mm halo); +++, high (9-12 mm halo); ++++, very high (≥13 mm halo)

Aflatoxin B1 reduction

The amount of AFB_1 bound in the supernatant was varied by considering bacillus isolates (figure 3). Generally, *Bacillus* strains were bound to 12.33 to 75% AFB_1. The AFB_1 binding of MA71 and MA82 increased significantly with continuing the incubation time ($P < 0.05$). However, at 1 h, the AFB_1 binding of the two isolates was significantly reduced, as compared with the first binding at 0 h. The strongest ability to detoxify AFB_1 for *bacillus* throughout the 24 h incubation was for MA82 (from 45% for 0 h to 75% for 24 h). The amount of AFB_1 bound depended on the time, with the most binding occurring after 24 h (75%). Among the two candidates, only the candidate MA82 had the strongest toxin binding.

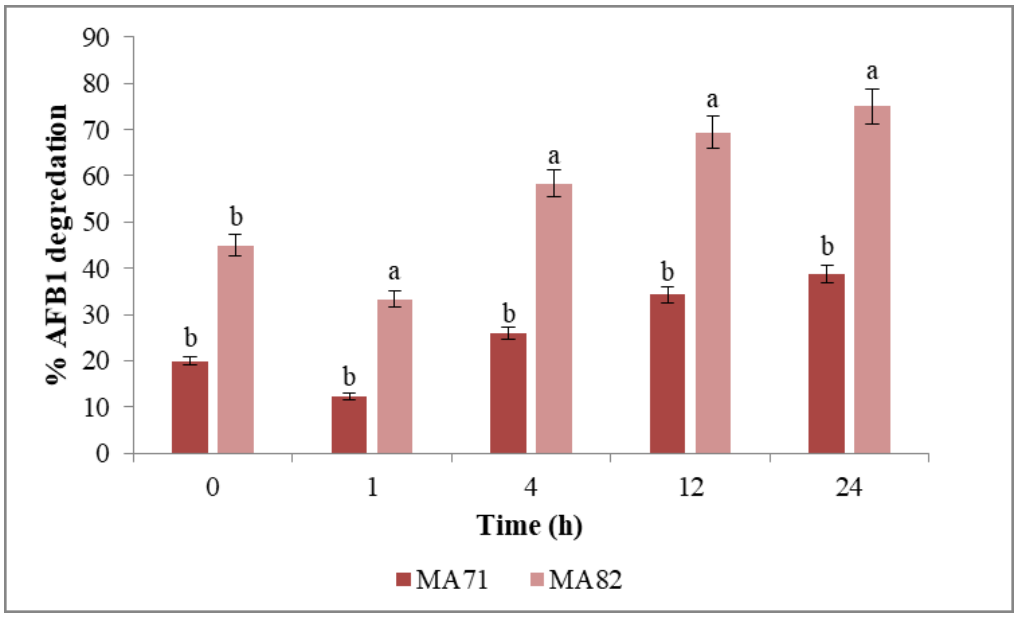

Figure 3.Aflatoxin degradation in supernatant after different incubation times. (Means ± SD)

PCR reaction and 16S rRNA analysis

The result of the PCR product on agarose gel is presented in Figure 4. The result showed that the MA82 belongs to the Bacillaceae family. Using the BLAST program, the nucleotide sequence was analyzed by the GenBank database. It was found that the MA82 was closely related to Bacillus. spp MBIA2.40. (Table 7)

Table 7. The result of 16SrRNA sequencing.

Description	Max score	Total score	Query over	E value	Ident	Accession
Bacillus sp. MBIA2.40 ribosomal RNA gen, partial sequence	1375	1375	85%	0.0	92.98%	KM438488.1

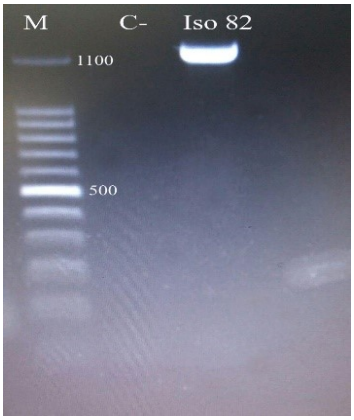

Figure 4. Agarose gel (2 %) electrophoresis showing band of genomic DNA.

Discussion

In this study, it is evaluated two bacillus strains from the gastrointestinal tract of avian for the potential probiotic and AFB_1 adsorption. This investigation showed that MA82 could mostly inhibit the growth of *E. coli* (O78:K80, O1:K1, O2:K1), *S. typhimurium* and *S. enteritidis,* as compared with others. This mechanism could be used for the production of bacterial substances such as bacteriocins; it is believed to be the main augment for probiotic strain screening (Hong *et al*., 2005). Thirty-one isolates of genes bacillus have been investigated in previous research (Latorre *et al*., 2016). It has been found that the various degrees of antimicrobial activity against different food-borne pathogens could be because of the capacity of some *Bacillus* to synthesize antimicrobial compounds (Latorre *et al*., 2016). Recent studies have shown that *Bacillus* probiotics can decrease specific pathogens in animal's feed (Bagherzadeh Kasmani, 2012). It was indicated that B. *amyloliquefaciens* LN as a probiotic candidate could inhibit the growth of *B*. *cereus* ATCC 11778, *B*. *cereus* ATCC 33019, and *L*. *monocytogenes* BCRC 15338. *B*. *cereus* and *L*. *monocytogenes* are well-known food-borne pathogens (Lee *et al*., 2017). A probiotic potential should be able to resist gut conditions such as gastric juice and bile salt (From *et al*., 2005). The first defence mechanism to cope with the ingested microorganisms is acid in the stomach, whereas bile salts in the duodenum reduce the survival of bacteria because lipids and fatty acids can encompass the bacterial cell membranes; so it is clear their highly susceptible to the destruction by bile salts (Jin *et al*., 1998). This study indicated that, MA82 and MA71were survived after incubation at the pH of 2, 3, 4 and 5 for 4 h or in the PBS containing 0.3% bile salt for the 4 h incubation.

One of the important characteristics of probiotic bacteria is its capacity for binding to epithelial cells and mucosal surfaces. In this study, toluene, xylene and n-hexadecane were applied as non-polar solvents. Their hydrophobic nature can be effective for interacting with the surface of microbes. Microbial adhesion to hexadecane is recommended as a marker for predicting the adhesion of microbial cells (Kiely and Olson, 2000) and at least 40% hydrophobicity is needed for the adhesion of a probiotic strain (Del Re *et al*., 2000). The current study found that both isolates had an average adhesion (over 40 %). One research has been reported that eight out of 12 isolates showed MATS < 40 % (Jain *et al*., 2017). It is suggested that aggregation properties, together with the coaggregation ability of bacterial strains, can impede the growth of potential pathogens used for the introductory selection of probiotic bacteria (Ferreira *et al*., 2011). The aggregation properties of MA82 and MA71 were similar. Coaggregation properties of MA82 with *Staphylococcus oureus* and *Salmonella enteritidis* were higher than MA71. Three isolates *bacillus* (MKSK-E1, MKSK-J1 and MKSK-M1) had 90% auto-aggregation over 3 hours (Lee *et al*., 2017). Biofilm could play a role in the protection against pathogens found in the intestine epithelium and thereby promote their survival and resistance. This study showed that both isolates have shown strong biofilm formation. Out of 31 *Bacillus* spp, 11 isolates have created a thicker and stronger adherent layer and it considered these isolates as superior biofilm formers (Latorre *et al*., 2016). In this research, the bacteria candidate was examined for their extracellular enzyme production of protease, amylase and lipase. These bacteria are known as inhibitors of pathogenic agents by the production of extracellular enzymes and improving feed digestion. In this research, two isolates were shown positive for protease, amylase and lipase which exhibit the clear zone on skim milk agar, starch agar and spirit blue agar. Researchers have shown that Bacillus amylase activity is 34 U/mL at 37°C/pH 7 (Sudharhsan *et al*., 2007). However, other research has reported that the amylase activity of *B*. *amyloliquefaciens* with an increased activity at 50°C was 72.5 U/mL (Abd-Elhalem *et al*., 2015). It was found that MA82 reduced the concentration of AFB_1 in PBS by 45% at 0 h, indicating that this strain might be capable of adsorbing AFB_1. The concentration of AFB_1 during

the incubation time was further reduced, receiving 75% after 24 h. It is indicated that the cell wall components of gram-positive bacteria were mainly responsible for the adsorption of mycotoxins; for example, it has been reported that the peptidoglycans *B. subtilis* cell wall could ensure fumonisin B1 adsorption (Niderkorn *et al*., 2009). Previous studies have shown that some *B. amyloliquefaciens* strains have the ability to degrade aflatoxins, ochratoxin, or zearalenone (Chang *et al*., 2015; Siahmoshteh *et al*., 2017).

For instance, it was reported that *B. amyloliquefaciens* UTB2 could inhibit *Aspergillus* growth and degrade aflatoxin B1 (Siahmoshteh *et al*., 2017). Also, another report has shown that B. *amyloliquefaciens* ASAG1 represented the degradation of ochratoxin, causing its carboxypeptidase activity (Chang *et al*., 2015). In future, it is suggested that *Bacillus sp*. MBIA2.40 was used in in-vivo conditions and its AFB_1 detoxification was evaluated in this state.

Conclusion

This research screened MA82, showing that it had antimicrobial activities against *Escherichia coli*, *Salmonella typhimurium*, and *Salmonella enteritidis,* provided resistance to the simulated gut condition, produced strong biofilm, had properties of hydrophobicity, aggregation and coaggregation and produced extracellular enzymes digestive. In addition, it had a stronger ability to detoxify AFB_1 rather than MA71. The degradation percentage of aflatoxin B_1 was 75% after 24 h. As well, Therefore, MA82 can not only be used as probiotics, but it can also serve as an adsorptive for aflatoxin B_1.

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Conflict of interest statement

The authors declare no conflict of interest.

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