ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Ability of *Bacillus Amyloliquefaciens* Isolated from Corn on mycotoxins degradation

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ABSTRACT

Somebacterial lactic acid strains and probiotics were used as microorganism detoxifying agents in a very few past years against harmful toxins specially mycotoxins. A novel strain of probiotic bacteria was isolated from Egyptiancorn grains and identified by 16srRNAassay. Bacterial strain was defined as *Bacillus amyloliquefaciens* strain MD1 16S, this strain was used as a commercial strain for animal feed, aquaculture, drain care, microbial concentrates,waste treatment, and septic. OchratoxinA(OCA) reducing property of *Bacillus amyloliquefaciens* strain MD1 16S bacteria could be used as a detoxification methodology that is most applicable for feed and food products. The strain of bacteria was tested for its fungal growth inhibition on three toxigenic fungi strains enclosed *Aspergillus sp*. strain, also for OCA and Zearalenone adsorption rate against other two different probiotics, *Streptococcus thermophilus* ATCC 4356 and *Lactococcus lactic spp lactic* ATCC 19435, which were used in dairy fermented products. Out of the tested strains, *Bacillus amyloliquefaciens* strain MD1 16S bacteria appeared to possess highest toxin removal rate of 88.1% and 61.3% for OCA and Zearalenone, respectively.

Keywords: Ochratoxin A – Zearalenone – *Bacillus* amyloliquefaciens – Corn

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ISSN: 0975-8585

INTRODUCTION

Corn is the third most important grain in the world yield and can be colonized by many plant pathogens from soil that reduce the quantity and quality of grain production. These grains are hosts of various fungi that can produce mycotoxins. Among these, fungi produce toxins associated with unwholesome impact on animal and human health. Populations of *Aspergillus* and *Fusarium* fungi were found in soil, crop residues, and the ambience of the corn farming [1]. These sources within corn farm may turn into potential inoculum provenance during corn outgrowth and may raise the inoculum at harvest level. Fungal diseases of crops are generally controlled by some cultural practices, fungicide implementation, and selection of resistant corn cultivars. The use of fungicides is argumentative practice that inherited unwanted environmental effects. Mean while mycotoxins soak up into body fluid from tiny viscous, the toxin accumulates in numerous tissues of the body as well as the tissues of liver and breathing system, renal and canal ductnervous, reproductive and immune system. Of whole body tissues, liver inclusive the major concentricity, around 10 double greater than within muscles [2].

A variety of detoxification techniques included in foods and feeds have been reported. These ones included physical ways such as using bentonite, hydrated aluminosilicate, and clay, also chemical ways as well as NaOCl, ammonia, and solvent extraction, then phytochemicals compounds like isothiocyanine, flavonoids, , and chlorophyll, finally biological agent uses as detoxifying factors as *Flavobacteriumaurantiacum*, whereas, actual, wide range, effective in cost and reliable mechanism owing to an outright detoxification of mycotoxins containing in human food or animal feeds square measure presently not out there [3]. The problem becomes troublesome for foods associated with human ever after the reassured handling and/or additives that would be permissible area unit difficult to demand. As an example; in overripe cheese area collective typically contaminated by aflatoxin M_1, and occasionally by aflatoxin B_1, cannot be agreeable any of previously additive or handling.

Probiotic bacteria, that grant health advantages while humans consumed it, are foremost lactic acid bacteria and its safety has been confirmed by its credible use for long times. Alongside its authenticity benefits, probiotic microorganisms safeguard versus food mutagens such as heterocyclic amines, nitroso-compounds and aflatoxin[4]. certian strains of lactic acid bacteria have been listed as aflatoxins and zearalenone binding agent from liquid media, milk and also from the gastrointestinal tract figuration a stabilized complex[5],[6]. Viable and heat or acid treated bacteria connected to toxin, amended of the binding respected to microorganisms and the strain type and it is linear relation within toxin, and bacterial concentration.

In the adsorption process, cell walls of bacteria connect to toxin with weak and non-covalent interactive joined with electrostatic attractiveness through lactinine like peptidoglycan, protein and polysaccharides [7]. Furthermore, Ambrosini*et al.,*[8]illustrated that; mycotoxun binding is a rapid operation done between microorganism and toxin; it had been done for 35 - 37°C, 6 – 7.5 as temperature and pH values, respectively. El-Nezami*et al.,*[9], reported the adsorption rates of toxin by *Lactobacillus lactis* diversified from 5.6% to 88% *invitro*; otherwise, Peltonen*et al*., [10] recorded increasing of binding ratio by using *Lactobacillus rhamnosus* GG, the binding ratio reached to 92% in chicks (*invivo* treatment), also by using two strains namely *L. rhamnosus* GG and *L. rhamnosus* Lc705, it had ability to remove AFM_1 from skim and whole milk as well as from phosphate buffer medium but with higher percentages, the removal proportion of aflatoxin $M_1$was found to rangefrom 18.8 to69.6% [5].

Several treatments had the ability to affect bacterial toxin adsorption, as an example, bacteria remediation with acid, or boiling, or autoclave treatment increased the binding ratio, otherwise, acid treatment was the most effective one that had the major binding values. El-Nezami*et al.,*[3] reported that; the exposed of probiotic cells to sonication, ethanol, irradiation, alkali, and ultra violet rays manifested either no impact or diminutive binding,the result of that study attributed the reason that heat as well acid alter surface properties of bacteria conducive to more highly toxin adsorption and lower desorption rates. One of the most important things for this way of toxification is binding of toxin by bacteria which decreases its adhesion to gastrointestinal tract, prevents biological systems from risks of toxin accumulation, at the same time it causes release those toxins out of the body [11].

A hopeful strategy to minimize mycotoxin accumulation in corn at harvest involved the biological interaction among toxigenic fungi and natural bio competitive agent. Use of bacteria and/or yeast to control microbial pathogens and pests of agricultural commodities has already been elaborated [12],[13]. In previous published studies, bacteria and yeast isolates were chosen to domination *Aspergillus* and *Fusarium* sp., [14],[15].

The aforementioned studies were all conducted on bio-control agents' strategy to control corn microbial hazards applied in the agro-ecosystem that they were originally isolated from. However, there are no studies of the impacts of selected bio-control agents on toxigenic fungi from agro-ecosystem native to them. Based on the addition of comparatively low numbers of native bacterial bio-control agents' strategy to corn crop at *invitro* trials, the present study tested the ability of corn non-native and native bio-control agents as bacterial strains to reduce corn infected by Egyptian fungal toxigenic species and its mycotoxins.

MATERIALS AND METHODS

Basic apparatus; chemicals and solvents:

Diagonal 115 VAC Rotary evaporator system –Shaker, Model EL680 —blender with high speed (15000 rpm) model JFSD – 100 grinding/subsampling mill for maize seed – EW-28615-00 – Micropipette (5 µl - 100 µl adjustable) – Cleanup cartridges, Romer labs. Inc. (USA). ZD20 four bowl Milli-Q water system (Millipore)– Chromatography column, 25 mm (i.d) X 300 mm length.

The HPLC grade solvents were obtained from Sigma (Germany). Other chemicals used through the study were of analytical reagent grade and obtained from Merck (Germany).

Media for isolation:

Czapekdox agar with 60µg/ml chloramphenicol (CDAC), and potato dextrose agar amended with 60µg/ml chloramphenicol (PDAC) were used for isolation of fungi from maize seeds.

Phosphate buffer saline:

Phosphate buffer saline solution was the medium for the toxin – bacterial binding reaction. Saline helps the complex formation. One tenth of mole of the buffer was prepared according to Lee, Y.K*et al*.[16], and the pH was adjusted to 7.3.

Standerd preparation :

Crystalline ochratoxin A (OCA) and zearalenone were from Sigma Aldrich, St Louis, MO, USA. Stock solutions (1mg/1ml) were prepared in methanol. Ochratoxin A stocks were stored at 20°C. Zearalenone Stock solutions (1mg/1ml) were prepared in acetonitrile/benzene (98/2). Methanolic working stocks were prepared by evaporating the acetonitrile/benzene solvent at 80°C, and reconstituting the solid in methanol.Aqueous working solutions of various concentrations were prepared by mixing alcoholic solution with phosphate buffer saline (PBS) (1ug/ml).The OCA and Zearalenonein reaction medium after centrifuging out bacterial-toxin complex was extracted and cleaned up according to AOAC method [17]. The dry film of the toxin was derivatized and derivative was quantitated with HPLC.

Cleanup using Immuno – affinity Columns

The extract was diluted to less than 5% acetonitrile using phosphate buffered saline. Then 18 mL of the resulting solution were loaded into the MycoSpin™ 400 Multitoxin column Art No. COCMY2400from Romer Labs®. The column was rinsed using 20 ml of water and eluted using acetonitrile. Acetonitrile was evaporated to dryness.

Identification of isolated bacteria by 16srRNA

a) DNA extraction

The genomic DNA of the strain was isolated according to Sambrook*et al*, [18]. Cells were collected from overnight LB cultures by centrifugation and re-suspended in 500 µl TRIS-EDTA-NaCl (TEN) buffer. Twenty five µl of 10mg/ml of lysozyme were added and the tubes were incubated at 37˚C for 30 min, followed by the addition of 75µl of 10% Sodium Dodecyl Sulphate (SDS) and the tubes were inverted gently several times till complete lysis. 3µl of 20 mg/ml of proteinase K were added and the tubes were incubated at 37˚C for one h. After incubation, 100 µl of NaCl (5M) were added followed by 800µl of phenol/chloroform: isoamyl alcohol (24:1) and the tubes were inverted several times and centrifugedat 12000 xgfor 10 min. The upper phase was transferred to a fresh tube and extracted once with chloroform. The upper phase was again transferred to a fresh tube and 0.7 volume of isopropanol was added and mixed gently and centrifuged again for 10 min. The supernatant was removed carefully and the pellets were washed with 1 ml of 70% ethanol. The pellets were collected by centrifugation at 12000 xgfor 5 min. The DNA was dried and dissolved in 100 µl TE buffer and stored at -80ºC.

b) PCR amplification

According to Messner*et al*., [19].The PCR amplification reactions were performed in a total volume of 100 µl. Each reaction mixture contained the following solutions 1µl DNA, 4µl 10 pmolforwed 16srDNA primer (5'-AAATGGAGGAAGGTGGGGAT-`3); 4µl of 10 pmol reverse 16srDNA primer (5'-AGGAGGTGATCCAACCGCA-`3); 10µl of 250 mMdeoxyribonucleotide 5'-triphsphate (N= A,T,G,C) (dNTP's); 10 µl PCR buffer, 3.5µl 25 mM $MgCl_2$ and 0.5 µlTaq polymerase and water was added up to 100 µl. The PCR-appratus was programmed as follows: 5 min denaturation at 94°C, followed by 35 cycles that consisted of 60 s at 95°C, 60 s at 58°C and 60 s at 72°C, and a final 10 min extension at 72°C. The products of the PCR amplification were analyzed by agarose gel electrophoresis (1%). Five µl PCR products were mixed with 1 µl of gel loading buffer. The mixture was loaded on agarose gels (1% w/v) in Tris-boric acid-EDTA (TBE) buffer (0.045 M Tris, 0.045 M boric acid, 0.001 M Ethylene diaminetetraacetic acid (EDTA), pH 8). The separation was carried out at 90 V for 40 min. The resulting DNA patterns were examined with UV light and photographed.

c) DNA-sequencing

The DNA was sequenced by the Genterprise Company (America).

Identification of bacterial isolate

For molecular identification of the isolated bacteria, Amplification of isolated bacteria DNA with general 16s primers for the 16S rDNA gave a single band of about 600 bp. The obtained fragment was sequenced. The sequences were analyzed with the BLAST program (EMBEL Gen Bank). The isolated strain showed a close relationship to the described strain, *Bacillus amyloliquefaciens* strain MD1. The complete identification of the isolated bacteria requires physiological and morphological tests in addition to molecular methods of DNA analysis. The systematic position of the bacterial isolate was determined by 16S rDNA sequence analysis. It showed a close relationship to the described strain, *Bacillus amyloliquefaciens* strain MD1. The complete identification of the isolated bacteria requires physiological and morphological tests in addition to molecular methods of DNA analysis.

Statistical Analysis:

Analysis of variance was executed, by program M Stat V.6 software, in this case significance at $P < 0.05$fordifference between the means were determined as fisher least significance difference test (LSD 0.05).

ISSN: 0975-8585

RESULTS AND DISCUSSIONS

Inhibition of fungal growth and mycotoxin production by isolated bacteria

New bacterial strain was isolate from Egyptian corn samples investigated in the present study; as during the fungal isolation from corn seed, swapping was done for search a strain that had a good effect to inhibit the toxigenic fungal growth, the swaps were spread on potato dextrose agar(PDA) to isolate some strains that have antimicrobial characters, each strain from the tens strains growth on PDA was tested to measure its inhibition against five strains of fungi producing toxins (, *Aspergillusflavus, Aspergillusochraceus, Aspergillusnigr, Fusariummoniliforme* and *F. oxysporum)*, of those isolated strains on PDA media there was one bacterial had better ability to inhibit the fungal growth of all fungal strains under the test condition, the bacterial strain was purified and identified using molecular biology method according to 16S rDNA to identifying and tested for its ability on fungal toxin production.

Different bacteria as well as their strains vary in their binding ability; this is why screening to select the proper strain is important. It was acutely approved that the accession of the activated bio-control microorganisms to YES media included *A. ochraceus* and *F. oxysporum* output inconsiderable suppression OCA secretion and mycelium development in a variance degrees as shown in Table (1). When *Bacillus amyloliquefaciens*strainMD1(Figure 1)*, Lactococcus lactic spp lactic* ATCC 19435*,* and *Streptococcus thermophilus*ATCC 4356 grew in a mixture the toxin binding rate of the mix was not enhanced. In other words, there was no synergistic effect on strain mixing.

Figure (1): Bacterial strain of *Bacillus amyloliquefaciens* strain MD1 isolated on agar media

Bacillus amyloliquefaciens strain MD1 isolated from corn grain were tested for its ability to inhibit the fungal growth of seven strains of fungi, *Aspergillus flavus, A. ochraceus , A. niger, A. carbonariusFusariummoniliforme, Penicillium spp.*and *F. verticillioides*. Bacterial strain showed inhibition stress on the seven types of toxigenic fungi on agar plate media (Figure 2).

Likewise, the dry weight of toxigenic fungi mycelia was found to decrease significantly in YES media containing the three types of those bacteria strains, as the inhibition test had been done in flask contained a liquid media against *Bacillus amyloliquefaciens*strainMD1 that were used in the present study had inhibition effect being the most powerful than the other two strains investigated.

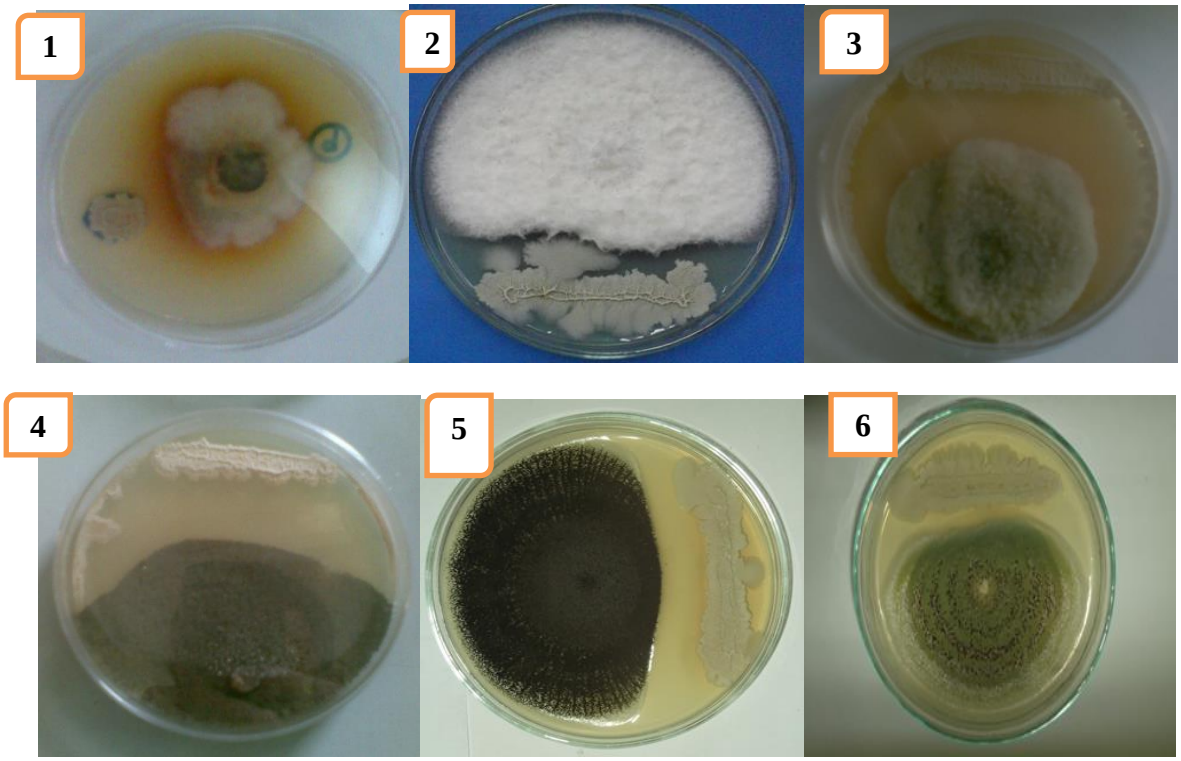
ISSN: 0975-8585

Table (1): Bacterial ability to reduce mycotoxin contamination from liquid media

Samples	Ochratoxin A (OCA)		Zearalenone (ZEN)	
	µg/kg	Reduction (%)	µg/kg	Reduction (%)
Control	180 ± 1.5	0.0	200 ± 2.0	0.0
Bacteria (1)	106.25 ± 0.34	40.97	143.95 ± 0.38	28.02
Bacteria (2)	55.42 ± 0.66	69.21	46.87 ± 0.33	76.57
Bacteria (3)	138.51 ± 0.47	23.05	153.54± 1.44	23.23

* Bacteria (1):*Lactococcus lactic spp lactic* ATCC 19435 – concentration at ($1.7*10^8$)
* Bacteria (2):*Bacillus amyloliquefaciens* strain – concentration at ($1.1*10^9$)
* Bacteria (3): *streptococcus thermophilus* ATCC 4356 – concentration at ($1.4*10^7$)
LSD = 1.99

Figure (2): Impact of *Bacillus amyloliquefaciens*strainMD1 on toxigenic fungal growth on PDA agar plate



Picture (1): *Fusariumverticillioides*, Pic(2): *F. moniliforme;* Pic(3): *A. Ochraceus*
Pic(4): *Penicillium spp.,* Pic(5): *A. niger, and* Pic(6): *A. parasiticus.*

Since it is important to screen large number of probiotics for their toxin adsorption, numbers of probiotic bacteria have been screened for their rate of OchratoxinA (OCA) and Zearalenone(ZEN) binding. Three strains one isolated from Egyptian corn samples and two commercial cultures were tested and results are given in Table (1). *Bacillus amyloliquefaciens* strain MD1 showed significantly (P> 0.05) better ability to remove OCA and ZENfrom PBS(69.21&76.57%, respectively) at 37°C than the other strains, followed by *Lactococcus lactic spp lactic* ATCC 19435 (40.97& 28.02%) and *Streptococcus thermophilus* ATCC 4356 was the lowest in binding (23.05% &23.23% for OCA and ZEN,respectively).These binding rates were with bacteria cell counts mentioned in Table (1) footnotes. However, using lower cell counts led to lower toxin removal rate.

However, the concentration of $1x10^6$ CFU/ml is recommended for probiotic products to show health benefits, therefore, the recommended cell load should be modified when probiotic is used for health benefits as well as detoxification. The ZEN binding by *Bacillus amyloliquefaciens* strain MD1 bacteria was less than its OCAbinding ability. For OCA and ZEN; the strain of *Bacillus amyloliquefaciens* strain MD1 comes as the greatest one forreduction inhibition followed by *Lactococcus lactic spp lactic* ATCC 19435 strain, the strain of *Streptococcus thermophilus* ATCC 4356 came as the lowest one of the three strains in OCA inhibition ratio.

Notwithstanding, inhibition ratio of *Lactococcus lactic spp lactic* ATCC 19435 for OCA toxin was moderated between the other two strains; but for ZEN, it was closed to *Streptococcus thermophilus* ATCC 4356 in toxin inhibition ratio.The binding ratios here were recorded as the least values than others types of bacteria that were usedsuch as *L. rhamnosus* GG (78%) [9].

***Mixing impact of bacterial strain on mycotoxin reducing*:**

In the experiment of mixing bacterial effective strains that were used as a method to enhance reduction ability of bacteria. The strain of *Bacillus amyloliquefaciens*strainMD1 was mixed by*Lactococcus lactic spp lactic* ATCC 19435, for OCA reduction ability, the new mixture had better ability thanthat of *Lactococcus lactic spp lactic* ATCC 19435 alone but it little bit enhanced the ability of *Bacillus amyloliquefaciens*strainMD1. Along with that; ZEN binding ability for the same mixture was more effective than use *Lactococcus lactic spp lactic* ATCC 19435 solo, but the result was not for from using *Bacillusamyloliquefaciens*strainMD1 alone.

The mixture of *Bacillus amyloliquefaciens*strainMD1and*Streptococcus thermophilus*ATCC 4356 did not changereduction ability of *Bacillusamyloliquefaciens*strainMD1 neither for OCA nor for ZEN toxin. Otherwise, the mixture of *Lactococcus lactic spp lactic* ATCC 19435 with and *Streptococcus thermophilus*ATCC 4356 had a good result for OCA reduction ability as well for ZEN reduction ability, the new reduction ratio was more better than use each of them separately. For OCA reduction ratio, it was changed from 23.05% for *Streptococcus thermophilus*ATCC 4356 and 40.97% for *Lactococcus lactic spplactic* ATCC 19435 to reached 45.41% for the mixture of the two strains. Along with that, ZEN reduction ratio also changed from 28.03% for *Lactococcus lactic spp lactic* ATCC 19435 and 23.23% for *streptococcus thermophilus*ATCC 4356 to reach to 30.2% for the mixture of the two strains, as it is showed in Table (2)

Table (2): Impact of mixing of bacterial strains on Mycotoxin Reducing

Bacterial strains Mix	Ochratoxin A (OCA)		Zearalenone (ZEN)	
	µg/kg	Reduction (%)	µg/kg	Reduction (%)
Control	**180 ± 1.5**	**0**	**200 ± 2.0**	**0**
(1) + (2)	**51.57 ± 1.61**	**71.35**	**43.34 ± 1.87**	**78.33**
(2) + (3)	**54.15 ± 0.79**	**69.91**	**46.6 ± 1.33**	**76.7**
(1) + (3)	**98.26 ± 2.44**	**45.41**	**60.41 ± 2.11**	**30.21**
(1) + (2) + (3)	**51.17 ± 0.91**	**71.57**	**41.1 ± 1.81**	**79.45**

* Bacteria (1):*Lactococcus lactic spp lactic* ATCC 19435 * Bacteria (2):*Bacillus amyloliquefaciens* strain
* Bacteria (3): *streptococcus thermophilus* ATCC 4356 LSD = 0.891

As for the mixture of three strains (*Lactococcus lactic spp lactic* ATCC 19435, *Bacillus amyloliquefaciens*strainMD1, and *streptococcus thermophilus*ATCC 4356) the reduction ratio for OCA toxin was not so far different from the result of using *Bacillus amyloliquefaciens*strainMD1 alone or use the mixture consisting of *Lactococcus lactic spp lactic* ATCC 19435 with *Bacillus amyloliquefaciens*strainMD1 (Table 1, 2). The same result was found in case of using *Bacillus amyloliquefaciens*strainMD1 alone or using a mixture of *Lactococcus lactic spp lactic* ATCC 19435 with *Bacillus amyloliquefaciens*strainMD1 to reduce ZEN toxin.

The effect of pH values on mycotoxin reducing power of the bacterial strain

Effect of pH values change on the efficiency of *Bacillusamyloliquefaciens*strainMD1 for mycotoxin reducing was studied, five values were used for the experiment as 3.2, 4.1, 5.7, 7.3, and 8.2 for determination of pH impact on bacterial ability to decrease mycotoxin amount in liquid media of phosphate buffer saline, acitic acid, chloric acid, and sodium hydroxide were used to adjust the pH at certain values. At all values except for pH = 7.3, the bacterial cells were found to die and the effect would not refer to its viability.

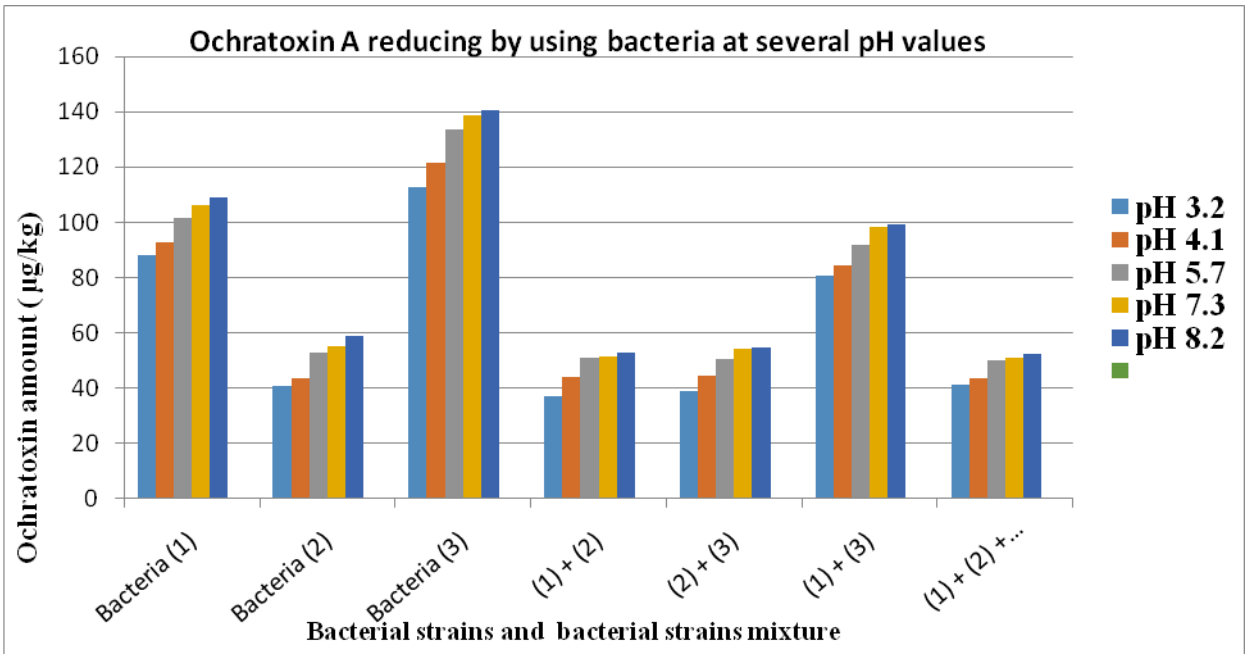
For OCA, the best degradation ratio was at pH equal to 3.2, in that case the bacterial cell was treated by chloric acid, and the reducing value was more highly even if for the value at pH equal to 4.1 was also treated with chloric acid. The degree of reducing change in OCA was not as the same in case of using acetic acid to adjust value of 5.7 pH. The decreasing ratio of OCA by using organic acid was less effective than using mineral acids. By increasing the pH values, the decreasing effect of bacterial exhibited higher values, likewise

ISSN: 0975-8585

in the alkaline pH of media where the bacterial strain decreasing efficient was less. According to the results that showen in Figure (3); mixing *Bacillus amyloliquefaciens*strainMD1 with either *Lactococcus lactic spp lactic* ATCC 19435 or with *Streptococcus thermophilus* ATCC 4356was not more effective than use *Bacillus amyloliquefaciens*strainMD1 as solo.

As the same like OCA; samples of ZEN toxin in media were treated by *Bacillus amyloliquefaciens*strainMD1 to determine its ability to reduce toxicity, ZEN decreasing values were higher at pH of 3.2 value, by increasing pH value of acidity to neutral direction the bacterial ability to decline toxin was limited. Low decreasing value of ZEN was at pH 7.3, and at alkaline condition with pH at 8.2 the decreasing ratio was at the lowest level. There was no considerable changes neither for use of *Bacillus amyloliquefaciens*strainMD1nor*Lactococcus lactic spp lactic* ATCC 19435nor with*Streptococcus thermophilus*ATCC 4356as mixed in variable pH values, nor for using the three strains as mixture at several pH values for ZEN reducing ratios. The results were comparing use of *Bacillus amyloliquefaciens*strainMD1 lonely or as several mixtures with other bacterial strains(Figure 4). All other factors such as temperature, toxin concentration, and incubation period were stable within the experiment.

Figure (3): Bacterial strains ability to reducing Ochratoxin A at several pH.



Bacteria (1):*Lactococcus lactic spp lactic* ATCC 19435, Bacteria (2):*Bacillus amyloliquefaciens* strain, Bacteria (3): *streptococcus thermophilus* ATCC 4356

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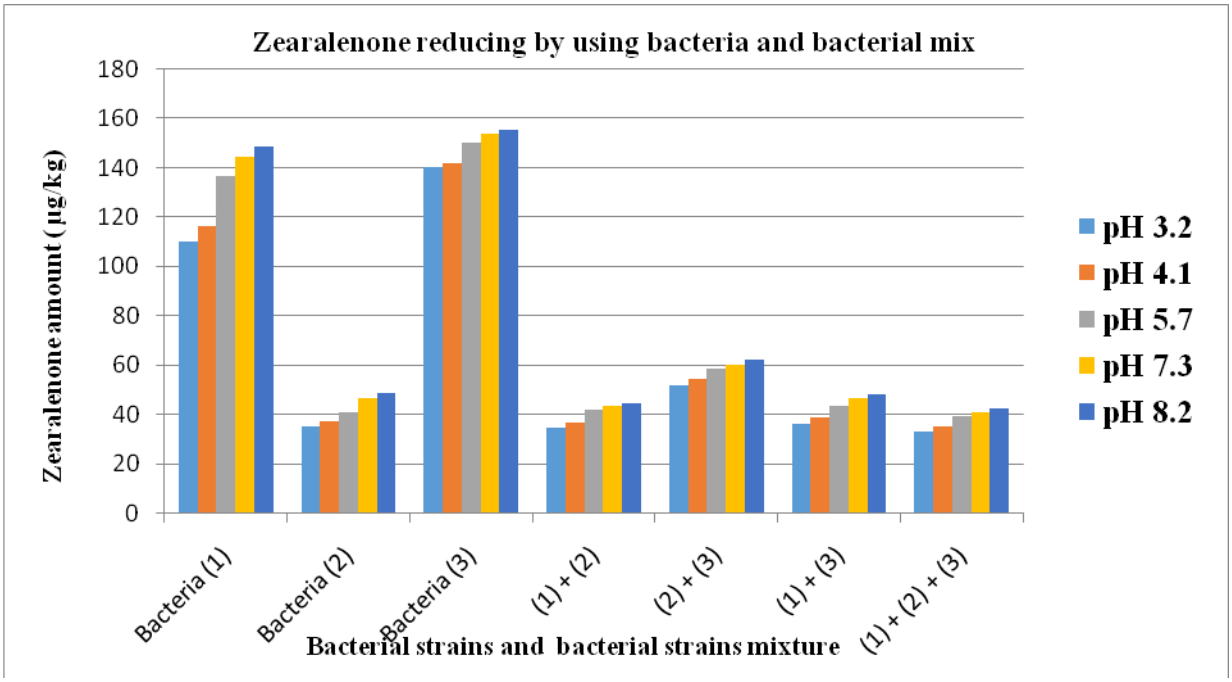
Figure (4): Bacterial strains ability to reducing Zearalenone at several pH.



Bacteria (1):*Lactococcus lactic spp lactic* ATCC 19435, Bacteria (2):*Bacillus amyloliquefaciens* strain, Bacteria (3): *streptococcus thermophilus* ATCC 4356

Getting along with Niderkorn *et al.,*[20]who studied binding of *Fusarium*mycotoxins by fermentative bacteria *invitro*, results showed that; by using of three bacterial strains, *Lactobacillus rhamnosus* strain GG ATCC 53103, *Lactobacillus delbruekii ssp. bulgaricus* R0149 and *Leuconostocmesenteroides* R1107, that were applied to decide detoxifying effect and the impact of pH for bacterial media on *Fusarium* toxins limitation, ZEN and FB_2 more efficiently removed than deoxinivalenol, nivalenol, and FB_1. At pH value equal to 4, the removal of fumonisinshad the highest degree and it reduced gradually at higher pH values. For ZEN, toxin removal, it was also influenced by pH for *Lactobacillus rhamnosus* strain GG ATCC 53103, *Lactobacillus delbruekii ssp. bulgaricus* R0149, but the differences were not as remarkable as that for the fumonisins.

On the other side; El Nezami*et al*., [3] found that; many factors could affected on bacterial strain ability to reduce aflatoxin B_1 one of these factors is the pH values, in that result pH in a range between 4 to 6 did not seem to share in disappearance of AFB_1, which was in contraindication to Megalla and Hafez [21] results who recorded that; pH may share in aflatoxin B_1 transformation to a less toxic form in acidogenous yogurt. comparable results were also reported by Rasic*et al*.,[22]who notified that in yogurt fermentation and milk acidified containing AFB_1 highly decreasing of toxin amount had been occured.

CONCLUSIONS

Ochratoxin A (OCA) and Zearalenone (ZEN) are hazard materials which could be found on food and feed materials, isolation of some microorganisms that found on cereal crops may had a good effect to decrease the hazard of mycotoxin contamination on cereal grains itself. *Bacillus amyloliquefaciens*strainMD1 was isolated and identified from corn grains, this strain of bacteria was able to reduce OCA and ZEN toxin amount in liquid media, it was also had a good impact on toxin producing fungi growth on agar media. In comparing with two other strains (*Lactococcus lactic spp lactic* ATCC 19435 and *streptococcus thermophilus*ATCC 4356) ability to reduce OCA and ZEN toxin amount, *Bacillus amyloliquefaciens*strainMD1 was the more effective one. Three bacterial strains mixing together were less effective than*Bacillus amyloliquefaciens*strainMD1 to reduce OCA and ZEN toxin from media. Changes of media pH values had a good effect on toxins reducing when the media being more acidity.

ACKNOWLEDGEMENT

This research was supported as part of project entitled Novel Integrated Strategies for Worldwide Mycotoxin Reduction in the Food and Feed Chains (MycoRed project), Coordinated by Antonio F. Logrieco, ISPA, CNR. And the project funded by program of FP7 funded Project, European Commission, European Union. Collaborative Project for Specific Cooperation Actions dedicated to international cooperation partner countries.

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