



Isolation, Screening and Identification of Swine Gut Microbiota with Ochratoxin A Biodegradation Ability

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ABSTRACT : The potential for ochratoxin A (OTA) degradation by swine intestinal microbiota was assessed in the current study. Intestinal content that was collected aseptically from swine was spiked with 100 ppb OTA and incubated for 6 and 12 h at 39°C. An OTA assay was conducted using the incubated samples, and it was found that 20% of the OTA toxin was detoxified, indicating the presence of microbes capable of OTA degradation. Twenty-eight bacterial species were isolated anaerobically in M 98-5 media and 45 bacterial species were isolated using nutrient broth aerobically. Screening results showed that one anaerobic bacterial isolate, named MM11, detoxified more than 75% of OTA in liquid media. Furthermore, 1.0 ppm OTA was degraded completely after 24 h incubation on a solid 'corn' substrate. The bacterium was identified by 16S rDNA sequencing as having 97% sequence similarity with *Eubacterium bifforme*. The isolation of an OTA-degrading bacterium from the swine natural flora is of great importance for OTA biodegradation and may be a valuable potential source for OTA-degradation enzymes in industrial applications. (**Key Words :** Ochratoxin A, Degradation, Isolation, Swine Intestinal Microbiota, Enzyme-linked Immunosorbent Assay)

INTRODUCTION

The global prevalence of mycotoxins in food and feedstuffs for human and animal consumption poses serious health hazards. According to the Food and Agricultural Organization (FAO), 25% of the world's commodities are annually affected by known mycotoxins. Among the mycotoxins of agricultural importance, ochratoxin A (OTA) is known to be a potent toxin having hepatotoxic, carcinogenic, nephrotoxic and immuno-reducing properties (Smith and Moss, 1985). Practical detoxification approaches that are applied to render contaminated feedstuffs free of toxins are categorized into three method groups: physical, chemical, and biological. Research indicates that the physical and chemical methods have certain limitations in that they are expensive, the nutritive value of the foodstuff is lost, or there is reduction in feed palatability (Varga and Toth, 2004). The biological method

as an alternative approach for degradation is considered to be the safest and most appropriate way of decontamination (Sweeny and Dobson, 1998; Bata and Lasztity, 1999). Biological degradation is the application of microbes or enzymes for the detoxification of mycotoxins of public and animal health concern.

Several microbes and enzymes have been reported to detoxify certain mycotoxins. The first was *Flavobacterium aurantiacum* with the ability to detoxify aflatoxins (Ciegler et al., 1996). Wegst and Lingens (1983) reported degradation of OTA by the aerobic bacterium *Phenylobacterium immobile*. Additionally, certain species belonging to the *Pleurotus*, *Bacillus* and *Acinetobacter* genera (Varga et al., 2005; Fuchs et al., 2008) and certain fungi belonging to *Aspergillus* (*A. fumigatus*, *A. niger*, *A. carbonarius*, *A. japonicus*, *A. versicolor*, *A. wentii* and *A. ochraceus*), *Alternaria*, *Botrytis*, *Cladosporium*, *Phaffia*, *Penicillium* and *Rhizopus* (*R. stolonifer* and *R. oryzae*) genera (Abrunhosa et al., 2002; Varga et al., 2005; Peteri et al., 2007) are able to degrade 95% of OTA *in vitro*, and some of them have been shown to have detoxifying properties in *in vivo* assays (Fuchs et al., 2008).

Rumen microbes have also been reported to degrade

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OTA, and *Bacillus* spp. possessing the gene for carboxypeptidase A have been implicated as the responsible organisms among rumen bacteria to degrade OTA (Upadhaya et al., 2009, Upadhaya et al., 2010). Carboxypeptidase present in *Phaffia rhodozyma* has been reported to degrade OTA (0.5-1.0 ppm) up to 90% levels (Peteri et al., 2007). Deberghes et al. (1995) reported carboxypeptidase A as the enzyme responsible for OTA detoxification, and the use of atoxigenic *A. niger* strains as the source of carboxypeptidases has been reported (Varga et al., 2000). In addition, Stander et al. (2000) reported that lipases could be obtained from *A. niger* strains that efficiently degrade OTA.

Since a significant fraction of food crops worldwide are contaminated with mycotoxins, including OTA, safer ways to decontaminate these foods are needed. Microbes or enzymes could be a practical way to reduce the levels of these contaminants. Thus, it would be of great interest to isolate bacteria and identify additional elements or enzymes that take part in the detoxifying process. The present study focused on screening and isolating microbes with OTA degradation ability from the intestinal microbiota of the highly OTA-susceptible swine host.

MATERIALS AND METHODS

Microbial sample collection from swine intestinal content

Immediately after the farm slaughter of fattening pigs (180 d old), portions of the intestine and their contents from two pigs were incised with a sterile scalpel and immediately placed in a sterilized Duran bottle. The bottle was closed tightly, put on dry ice and transferred to the laboratory. The intestinal digesta was withdrawn into a sterile 100 ml conical flask and filtered through eight layers of cheesecloth. The filtrate was flushed with CO₂ using a gassing apparatus and placed in an anaerobic chamber (COY Laboratory Products Inc, Grass Lake, Michigan, 49240, USA) for further experimentation.

OTA degradation assays

Anaerobic swine intestinal digesta (10 ml) was mixed with 50 ml sterile 100 mM PBS (GIBCO, Invitrogen Corporation 1600 Faraday Ave. Carlsbad, CA 92008) prepared anaerobically. Triplicate samples (5 ml) of the mixed solution were placed in screw-capped sterile Hungate tubes fitted with butyl rubber stoppers and spiked with 100 ppb OTA. The cultures were incubated for 6 h and 12 h at 39°C in a shaking incubator. After 6 h and 12 h incubation, an OTA assay of the samples was conducted using a commercially available ELISA kit (Romer Labs Inc., 3791 Jalan Bukit Merah Singapore).

Isolation of bacteria from swine intestinal fluid

Both aerobic and anaerobic bacteria were isolated from the swine intestinal digesta. The intestinal digesta were serially diluted in sterilized PBS and a 200 µl aliquot from each dilution was spread on M 98-5 (Salanitro et al., 1974) and nutrient agar media (Difco™ Becton, Dickinson and Company Sparks, USA) for anaerobic and aerobic isolations, respectively. Anaerobic cultures were incubated in an anaerobic chamber. Plates were incubated at 39°C for 24 h. Single colonies were picked and streaked onto their respective agar media three subsequent times to ensure the isolates were pure. Isolates were stored in liquid media containing 20% glycerol in cryovials at -80°C.

Screening of isolates for OTA detoxification

After the isolation of bacterial strains using either the anaerobic or aerobic technique, isolates were screened for OTA activity by transferring fresh bacterial inocula into M 98-5 media supplemented with 100 ppb OTA and incubated for 12 h at 39°C. Media supplemented with OTA but without cells was used as a negative control. Sampling for OTA degradation was conducted at 0 and 12 h by extracting the sample with 100% HPLC grade methanol (Sigma Aldrich Spruce Street, St. Louis, USA). The OTA degradation assay was conducted using a commercially available ELISA assay (Romer Labs Inc., 3791 Jalan Bukit Merah Singapore). Screened isolates were prioritized by ranking on OTA degradation ability. Prioritized bacterial isolates were again measured for OTA activity in triplicate, and isolates with the best OTA degradation activity were selected for further characterization.

Media modification and OTA degradation kinetics using ELISA

Originally, the media used to isolate anaerobic bacteria was M 98-5. Since the growth of the screened isolate was not satisfactory, a modified M 98-5 medium that was supplemented with vitamin K (0.5 µg/ml) and yeast extract (5 mg/ml) was used. Frozen cultures (100 µl) were inoculated into fresh modified 5 ml media at 39°C for 12 h and 500 µl of this fresh culture was inoculated into numerous 4.5 ml modified medium cultures supplemented with 100 ppb OTA for 0, 4, 8, 12 and 24 h incubations at 39°C with agitation. Sampling for the OTA degradation assay was done at the different incubation times by extracting each sample with 100% methanol (HPLC grade) and OTA activity was measured using a commercially available ELISA kit (Romer Labs Inc., 3791 Jalan Bukit Merah, Singapore). Furthermore, to assess OTA activity at this higher concentration OTA kinetics were assessed for each isolate with media spiked with 1.0 ppm OTA.

OTA degradation confirmation using HPLC

The OTA-degrading isolate was cultivated in 10 ml modified medium spiked with 1.0 ppm OTA in triplicate. Samples (6 ml) taken before and after 8, 12, and 24 h incubation were centrifuged at 10,000 rpm (Supra21K, High speed refrigerated centrifuge, Hanil, Science Industrial Co., Korea) for 5 min and the supernatant was extracted in a 1:1 ratio with ethyl acetate (Sigma Aldrich, St. Louis, MO, USA) and then vortexed. Vortexed samples were centrifuged at 10,000 rpm for 5 min and 5 ml of the supernatant was collected and dried using a rotary evaporator (EYELA N-N Rikakai Co., Ltd. Koishikawa, Bunkyo-ku Tokyo, Japan) and finally dissolved in 1.0 ml HPLC grade methanol (Sigma Aldrich, St. Louis, MO, USA) for HPLC injection. Samples (20 μ l) and standards (10 ppb, 50 ppb and 100 ppb) were injected and detection was conducted using a fluorescence detector (RF2000, Dionex, Sunnyvale, CA, USA) with excitation at 333 nm and emission at 460 nm. The peak for each standard was integrated and identified and the peak area was plotted against concentration to give a calibration curve. For unknown samples, the peaks were integrated and identified, and the peak areas were related to a concentration from the calibration graph. The OTA linearity in the working standard solutions at four concentration levels of 10, 50, 100 and 1,000 ng/ml was excellent, as shown by the correlation coefficient ($r = 0.999$). The limit of detection was 5 ppb.

The solvent system used was a 1:1 ratio of acetonitrile to water (3 DW) with 1% acetic acid. The flow rate was 1.0 ml/min.

Fractionation of cells for OTA degradation study

Different bacterial cell fractions were prepared to include i) whole cells (cells+broth), ii) broth only, iii) cell free extracts dissolved in sterilized PBS, iv) PBS only and v) autoclaved cells. The cell free fraction was obtained by centrifuging the bacterial culture at 20,000 \times g for 20 min at 4°C. To obtain cell free extracts, cells were harvested by centrifugation at 4°C at 6,000 \times g (Supra K21, High Speed refrigerated centrifuge, Hanil Science Industrial Co, Korea) for 20 min. The pellets were washed twice and resuspended in PBS (100 mM; pH 7.4) to prepare for cell rupture (3 ml buffer per gram cell mass). The cells were disrupted by triplicate sonication treatment (Vibra-Cell, Sonics & Materials Inc., Newtown, CT, USA). Cell disruption was conducted on ice to ensure the low temperature conditions required for enzyme stability. The disrupted cell suspension was centrifuged at 20,000 \times g for 20 min at 4°C. The supernatant was aseptically filtered using 0.2 μ m sterile cellulose pyrogen-free disposable filters (Advantec, MFS Inc., Japan)

OTA degradation by different fractions

To assess OTA degradation by different bacterial fractions, 20 ml of each fraction was supplemented with 100 ppb OTA and triplicate 6 ml samples were distributed to Hungate tubes and incubated for 24 h at 39°C with agitation. Autoclaved cells in medium with OTA, broth (medium) with OTA and PBS with OTA were used as the negative control. Sampling for the OTA assay occurred at 0 and 24 h and assay was done using ELISA Kit.

Assessment of OTA degradation on a solid substrate

About 3.0 g of ground corn were added to a 125 ml serum bottle (in triplicate), flushed with CO₂ gas to maintain anaerobic conditions, and autoclaved at 121°C for 20 min. The sterilized grains were then spiked with 2.0 ml of 1.0 ppm OTA and then inoculated with 2.0 ml of isolated culture inocula in an anaerobic chamber. The inoculated substrate was stirred thoroughly with a sterilized wooden stick to ensure even distribution. Sampling for the OTA assay was conducted at 0 and 24 h of incubation and the analysis was done using ELISA kit. The control experiment consisted of a non-inoculated OTA-spiked corn solid substrate under anaerobic conditions.

Isolate identification using molecular and conventional techniques

The MM11 isolate was cultivated on fresh M98-5agar media and a single colony was chosen to observe its colony characteristics and morphological structure via a Gram stain. Molecular characterization of MM11 was conducted via 16S rDNA sequencing. The 16S rDNA gene was PCR amplified using the following universal primers; 27f: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492r: 5'-TACGGYTACCTTGTTACGACTT-3'. The PCR conditions were as follows: one cycle of initial denaturation at 95°C for 15 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min. PCR products were separated on a 1% agarose gel (Bio-WORLD, Dublin, OH, USA) containing ethidium bromide. PCR products were excised from the gel and purified using the QIAquick spin gel extraction kit (Qiagen, Korea Ltd Woolim-Lions Valley 371-28 Gasandong, Gumchungu Seoul 153-786) as per manufacturer's instructions. Purified PCR product was cloned into the pGEM-T Easy vector (Promega Corporation, 2800 Woods Hollow Road, Madison, USA) and resulting clones were used to transform *Escherichia coli* DH5 α . The recombinant plasmid was extracted from transformants using a plasmid DNA purification kit (iNtRON Biotechnology, Jungwon-Gu, Seongnam-Si, Gyeonggi-do, 462-120, Korea) as per manufacturer's instructions. DNA concentration was

measured using a NanoVue spectrophotometer (GE Life Sciences, Piscataway, NJ, USA). Scanning Electron Microscopy was done at NICEM, Seoul National University (www.nicem.snu.ac.kr) to determine the morphological characteristics of the isolate MM11.

Sequence analysis and phylogenetic analysis

The 16S rDNA sequence was determined by cycle sequencing. All reference sequences were obtained from GenBank database in NCBI (<http://blast.ncbi.nlm.nih.gov/Blast>) Sequences were aligned using a multiple sequence alignment tool (ClustalX (1.8)). A total of 20 sequences were obtained from NCBI BLAST searches, and were used together with the MM11 sequence to generate a phylogenetic tree. The sequences were aligned manually using Se-Al v. 2.0a11 alignment software (Rambaut, 2002). The aligned sequences were exported in NEXUS format. Phylogenetic trees were inferred from the optimized alignment by distance analysis using PAUP 4.04a software. *Eubacterium bifforme* was used as the outgroup for all analysis. Distance and branching order was performed using the neighbor joining method (Saitou and Nei, 1987).

Sample preparation and extraction for ochratoxin A analysis

Extraction of OTA from liquid media : OTA-spiked swine intestinal fluid samples or bacterial isolate cultures in liquid media were centrifuged at 13,000 rpm (Centrifuge 5415D Eppendorf) for 1 min. A 300 µl supernatant aliquot was added to a microfuge tube and mixed thoroughly with 700 µl 100% HPLC grade methanol (Sigma, Aldrich, Germany) via vortex for OTA extraction. Samples that were not immediately analyzed were stored at -20°C until analysis. Extracted OTA samples were diluted with 70% methanol and the OTA assay was conducted using the commercially available Agra Quant[®]Total Ochratoxin Kit (4 to 40 ppb) (Romers Labs).

Extraction of OTA from substrate : A 15 ml aliquot of 70/30 (v/v) methanol and distilled water was used as solvent to extract ochratoxin from 3.0 g of corn inoculated either with or without MM11 and supplemented with 1.0 ppm OTA or to a glass slide spiked with 500 µl of 1.0 ppm OTA as a substrate control. The sample was then vortexed for 3 min, allowed to settle, and then filtered using Whatman No.1 filter paper (Whatman International Ltd. Maidstone England). The filtrate was collected and analyzed for OTA similarly to that of liquid media.

OTA sample assay using ELISA : One hundred microliters of the Agra Quant[®]Total Ochratoxin Kit standards (0, 4, 10, 20, and 40 ppb) as well as samples to be analyzed were mixed with 200 µl conjugate in individual dilution wells. Next, 100 µl from each dilution well was

transferred to a respective antibody-coated microwell. After 15 min incubation at room temperature, each well was washed 5 times with distilled water, tapped dry using several layers of absorbent paper, and 100 µl of enzyme substrate was added to each well and allowed to incubate for an additional 5 min. Stop solution (100 µl) was then added and intensity of the resulting yellow color was measured optically using a VERSA microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm. The total incubation time of the test kit assay was 20 min. The detection limit of the kit was 2 ppb.

A calibration curve of absorbance versus concentration of the standards was obtained using the linear least squares regression procedure. Absorbance values obtained from the plate reader were interpolated to the Romers lab[®] data reduction spread sheet for the calculation of OTA concentration in ppb. The obtained ppb value was then multiplied by a factor of 2/3 as the original measured sample (300 µl) was diluted with 700 µl 100% methanol, giving a dilution factor of 10/3. In addition, the standards were prediluted by a factor of 5 as indicated in the manufacturer's instructions (Romers Labs Singapore Pte, Ltd). As such, for the final ppb values obtained, a total dilution factor of $(10/3) \times (1/5) = 2/3$ was taken into consideration. This dilution factor was not used in the case of solid substrate values.

Statistical analysis

Data generated were subjected to an analysis of variance (ANOVA) using SAS software (2002, SAS Inst. Inc., Cary, NC, USA). Differences among the means were tested using a least significant difference procedure (SAS, 2002). Values $p < 0.05$ and $p < 0.001$ were considered significant and highly significant, respectively.

RESULTS

Assessment of OTA degradation ability of swine intestinal fluid micro-organisms

The intestinal digesta that were mixed with sterilized PBS spiked with 100 ppb OTA and incubated for 6 and 12 h showed that OTA was degraded by about 20% though not significant as measured by ELISA (Figure 1).

Isolation and screening of bacteria from swine intestinal fluid

Bacterial isolation from swine intestinal content was conducted aerobically and anaerobically. A total of 28 anaerobic and 45 aerobic bacteria were isolated. From the total 28 anaerobic isolates, 9 isolates had OTA degradation activity ranging from 1 to 77% degradation in liquid medium supplemented with 100 ppb of OTA (Table 1). No

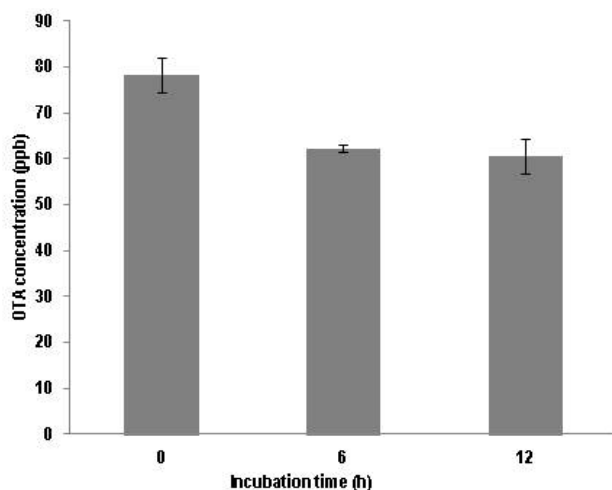


Figure 1. OTA degradation (100 ppb) in swine intestinal fluid. The OTA assay was assessed by ELISA.

aerobic isolates showed OTA degradation activity.

Growth and kinetics of OTA degradation by MM11 in modified liquid media

Modified M 98-5 medium, with the addition of yeast extract and vitamin K, led to better growth of the MM11 isolate with optical densities up to 0.45 or 10^5 cells/ml (data not shown). OTA degradation using ELISA was highest ($p < 0.001$) after 24 h incubation according to the kinetic study (Figure 2). OTA degradation by MM11 was also confirmed by HPLC assay. Our findings showed that OTA (1 ppm) was completely degraded ($p < 0.001$) at 24 h of incubation (Figure 3).

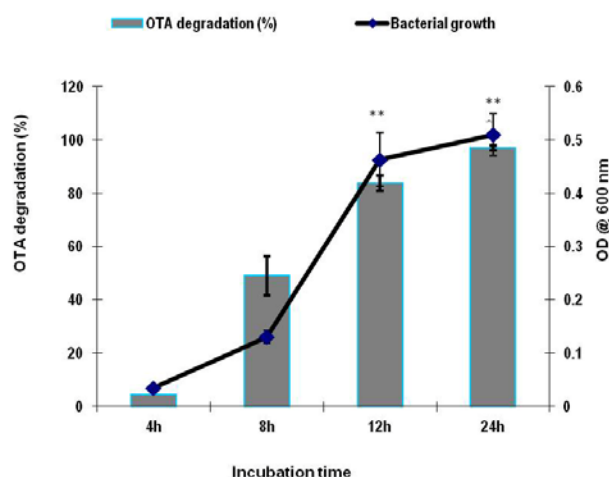


Figure 2. OTA degradation (%) and growth of isolate MM11 incubated in modified M 98-5 medium containing yeast extract and vitamin K at different incubation times. The double asterisk indicates results are highly significant ($p < 0.001$). OTA concentration was 100 ppb (ng/ml). OTA degradation assay by ELISA.

Table 1. Ochratoxin A (100 ppb) degradation ability of anaerobic isolates from swine intestinal contents in duplicates using ELISA

Ranking	Name	Degradation (%)	SEM
1	MM11	77.1	0.54
2	MM19	9.0	0.04
3	MM27	6.0	3.53
4	MM16	5.5	0.04
5	MM20	4.9	0.47
6	MM12	4.4	0.16
7	MM26	3.34	0.69
8	MM18	2.7	0.55
9	M1	1.9	0.17

Identification of potential isolates using molecular and conventional techniques

PCR amplification of the MM11 16S rDNA gene produced a 1,505 bp product. Sequence of the PCR product was compared to 16S rDNA sequences available in GenBank using the BLAST program (www.ncbi.nlm.nih.gov/BLAST). Sequence from the MM11 isolate showed 97% similarity with *Eubacterium bifforme*, as determined in phylogenetic analysis. Gram staining showed that the organism is Gram-positive. Microscopic examination revealed a coccus-shaped organism arranged in chains as seen in SEM images (Figure 4).

OTA degradation by cell fractions

Different cell fractions from isolated MM11 were examined for OTA degradation ability. It was found that cell free extracts after cell lysis had the highest OTA degrading ability ($p < 0.001$) followed by whole cells (Figure 5). No degradation was observed in autoclaved cells, medium only and PBS, which served as the negative control.

OTA degradation on a solid substrate

Autoclaved ground corn spiked with 1.0 ppm OTA and a subsequent inoculation with strain MM11 was incubated for 24 h at 39°C and OTA levels were assessed by ELISA. After 24 h incubation, almost 100% of the OTA was degraded ($p < 0.001$, Figure 6). However, 26% of the OTA was degraded in the negative control (corn without the added MM11 isolate) and 11% was degraded from a glass slide spiked with 1.0 ppm OTA without the MM11 isolate.

DISCUSSION

The contamination of agricultural products with mycotoxin has adverse effects on human and animal health throughout the world. Attempts to eliminate mycotoxins from contaminated products by physical and chemical methods have limitations (Scot, 1991; Scot, 1996) due to expense, and the loss of both nutritive value and palatability.

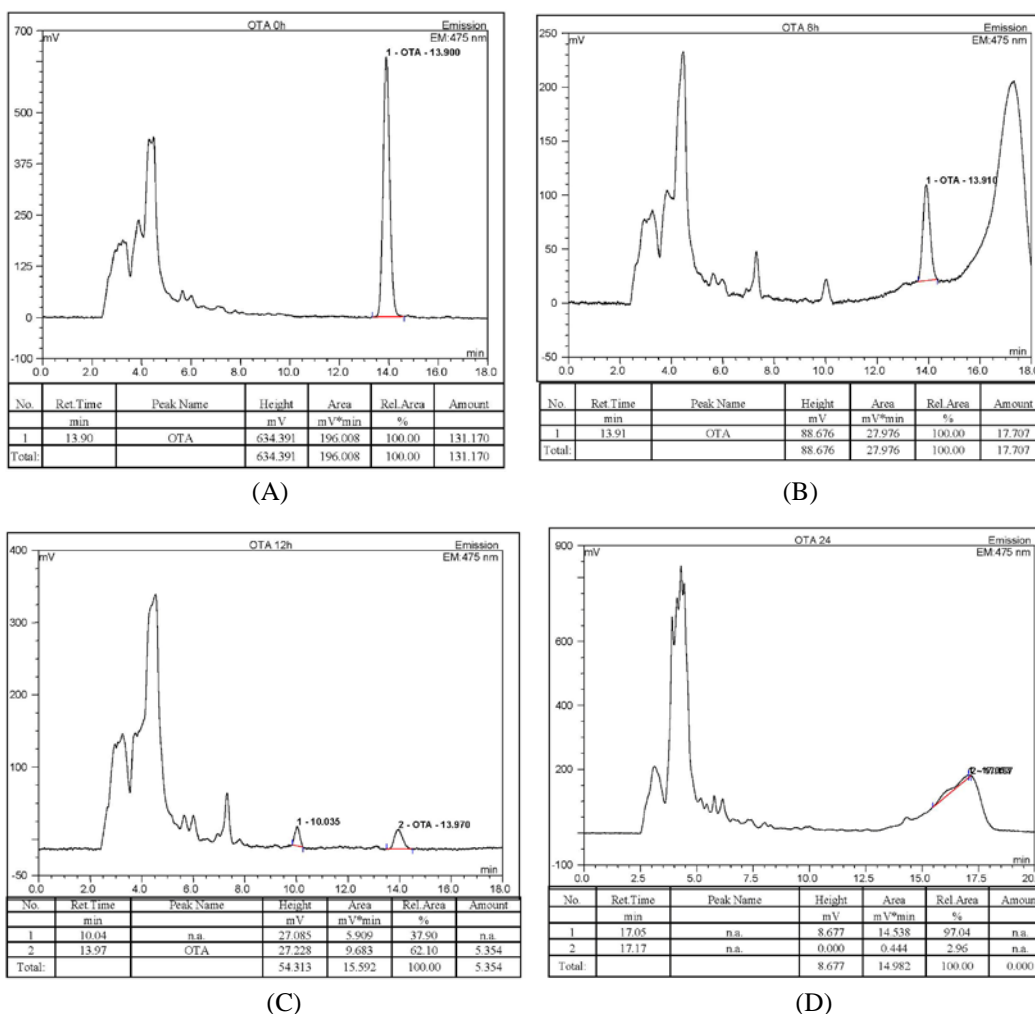


Figure 3. HPLC profile of OTA degradation by isolate MM11 at A) 0, B) 8 C) 12 and D) 24 h incubation. OTA concentration was 1 ppm (1 µg/ml).

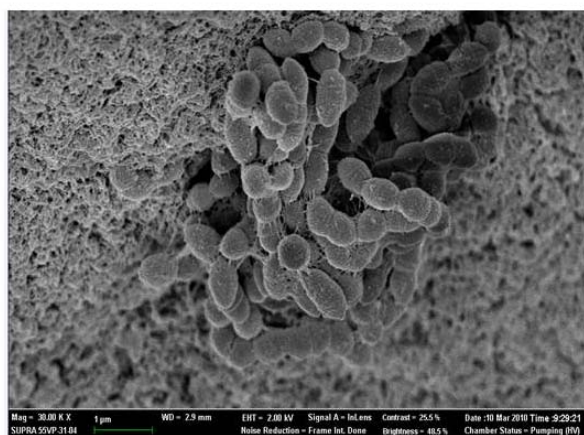


Figure 4. Scanning electron microscopy of isolate MM11.

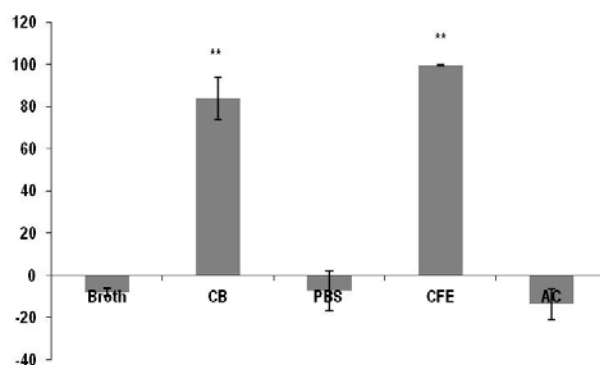


Figure 5. OTA degradation activity (%) in different cellular fractions of isolate MM11 after 24 h incubation. The OTA assay was conducted via ELISA. CFE; cell-free extract, CB; cells+broth, PBS; Phosphate Buffered Saline (control), AC; (autoclaved cells+medium) and Broth (control). The concentration of OTA was 100 ng/ml (ppb). The double asterisk indicates results are highly significant ($p < 0.001$).

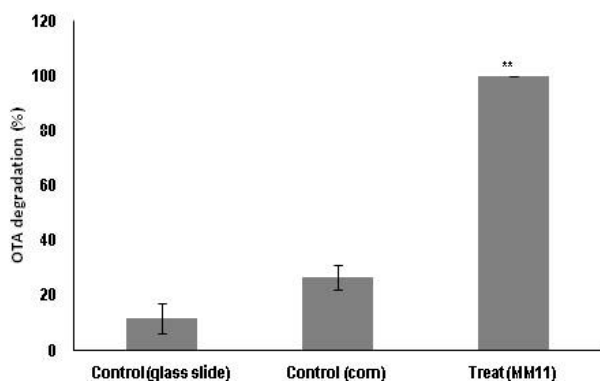


Figure 6. OTA degradation (%) on solid substrate (corn) spiked with 1.0 ppm OTA. Degradation assay done by ELISA. The double asterisk indicates results are highly significant ($p < 0.001$).

Thus, a biological approach is considered to be an alternate method that involves the application of microbes or enzymes (Sweeney and Dobson, 1998; Schatzmayr et al., 2006). Several studies have demonstrated the OTA-degrading activity of microbiota from the mammalian gastrointestinal tract, including the rumen of cow and sheep (Hult et al., 1976; Galtier et al., 1976; Xiao et al., 1991), and the intestine of rats (Madhyastha et al., 1992). This led us to investigate and isolate bacteria from swine intestinal fluid. Whole intestinal fluid from swine was used to investigate its capacity for OTA degradation. Other reports have indicated that gut microbes from monogastric animals as well as other mammalian guts contain OTA-degrading capacity. For instance, microbes living mainly in the intestines of rats (Madhyastha et al., 1992) and bacteria from swine including *Eubacterium callanderi* from the large intestine and *Eubacterium ramulus* from the small intestine were reported to be OTA-degrading strains, degrading almost 100% OTA over a 6 h incubation (Schatzmayr et al., 2006). In this study, one isolate designated as MM11, among the screened isolates had the highest OTA degradation level of 77% initially in liquid medium supplemented with 100 ppb OTA. However with the modification of medium, degradation was increased and OTA degradation was maximal after 24 h of incubation in liquid media. It is possible that the enzyme required to degrade OTA reaches its maximum concentration at 24 h of incubation. HPLC assay also confirmed that this isolate had high OTA degrading capacity. The potential isolate from swine gut was found to have 97% sequence similarity with *Eubacterium bifforme*. Schatzmayr et al. (2006) also reported that *Eubacterium callanderi* from the large intestine of swine and *Eubacterium ramulus* from the small intestine of swine were OTA-degrading strains, degrading almost 100% OTA over 6 h incubation. Based on the OTA degradation by different fractions of cells prepared from the

isolate, the highest OTA degradation was seen in cell free extracts. No degradation was observed in PBS supplemented with OTA and medium containing autoclaved cells supplemented with 100 ppb OTA, which served as the negative control, indicating that the enzyme responsible for degradation is cell bound. Peteri et al. (2007) also reported that the OTA degrading enzyme was associated with cell lysates in a yeast isolate.

Solid state fermentation holds tremendous potential for the production of enzymes. It can be of special interest in processes where the crude fermented product may be used directly as the enzyme source. In addition to conventional applications in food and fermentation industries, microbial enzymes have attained a significant role in the biotransformation of mycotoxins and other bioactive compounds. This system offers numerous advantages over liquid fermentation systems, including high volumetric productivity (Doelle et al., 1992). In the current study, OTA degradation was also assessed on a solid substrate. In solid-state fermentation, 100% of the OTA was degraded after 24 h of incubation with the isolate MM11, while the negative control showed 26% OTA loss. This could be due to some element from corn itself that led to OTA reduction. To ensure that the spiked corn incubated under anaerobic conditions in the medium without a bacterial inoculum could degrade OTA to some extent, an experiment with an OTA-spiked glass slide was used as a simulation for a solid substrate followed. In this case, only 11% of the OTA was lost, indicating that the corn itself had some element to degrade OTA under anaerobic conditions.

Microbiota from the swine intestine was found to have higher OTA degradation capacity. The anaerobic isolate MM11 has the highest OTA degrading potential in both liquid media and in the solid substrate fermentation state. Since OTA was degraded by the isolate on the corn substrate in the *in vitro* experiment, this isolate could contribute to the decontamination of OTA in other agricultural products. In solid-state fermentation, 100% of the OTA was degraded after 24 h of incubation with the isolate MM11, while the negative control showed 26% OTA loss.

IMPLICATIONS

The MM11 isolate might provide a source of enzymes that could be used for the detoxification of OTA contaminated feedstuffs. Anaerobic microorganisms isolated from animal guts are generally suitable for the development of feed additives that will act in the intestines of the targeted animals. Survival and adaptation of the microorganisms in the animal gut are key factors for successful detoxification.

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