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Research Article

BIOLOGICAL APPROACH TO DEGRADE MYCOTOXIN-AFLATOXIN B_1

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ABSTRACT

The Aflatoxin $B_1$is one of the most carcinogenic compound, commonly found in cereals and grains and bringsignificant threats to food and feed industry including animal production. The aimof this research was to look for aflatoxin B_1 degradative bacteria from soil samples and oil seeds. Among a collection of degradative bacteria isolates, the strains IFS2, IFS3, UISF5 and UISF7 were selected based on its ability to utilization of Aflatoxin B_1at concentration of (2µg/mL) as a sole carbon source. Thin layer chromatographyand Spectro-densitometric methods were employed to extract the residual Aflatoxin B_1 concentrations qualitatively and quantitatively. Bacillus sp. IFS2 had strong ability to degrade the highly toxic Aflatoxin B_1 where the degradation percentage of IFS2 culture supernatant degrade 61% AFB1 at48 hrs. of incubation period compared to 46% and 51% by cell extracts and viable cells, respectively. The results implied that aflatoxin B_1 degradation was mainly noticed in the cell supernatant than cell extractand cells culture. The cell supernatant was characterized by considerable activity at wide range of temperatures and P^H. The morphological and biochemical studies of identification indicated that the strains IFS2, IFS3, UIFS5 and UIFS7 belongs to Bacillus Sp. Pseudomonas Sp. Lactobacillus Sp. and Serratia Sp. Respectively. Based on the efficiency of degradation, Bacillus Sp. IFS2 characterized by 16S rRNA sequencing and the results indicated that IFS2 belongs to Bacillus thuringiensis and 626bp nucleotide sequence was provided with a GenBank accession number MG407658. Biosafety results indicated that Aflatoxin B_1 detoxification is an enzymatic and extracellular enzyme had the high ability to detoxify.*Bacillus thuringiensis*, was reported first time in the degradation of Aflatoxin B_1 by this research work.

INTRODUCTION

Aflatoxins are a group of closely related heterocyclic and toxic secondary metabolites produced predominantly by filamentous fungi viz., *Aspergillus flavus* and *Aspergillus parasiticus* (Yu *et al*., 2002*)*. Aflatoxin contaminates a vast array of food and agricultural commodities such as cereals, nuts, dried fruits, coffee, cocoa, spices, oil seeds, dried peas and fruits under different environmental conditions (Turner *et al*., 2009: Reddy *et al*., 2011). The contamination of these oil seeds occurred during the stages of growing, harvesting, storage, transporting and processing. Aflatoxin contamination of foods and feeds has gained global importance because of its deleterious effects on human as well as animal health (Okoli *et al*., 2006). The economic consequences of mycotoxin contamination are profound, as the crops contaminated with prominent levels of mycotoxin are often destroyed (Fakruddin *et al*., 2015). The reported outbreaks of aflatoxicosis in man were due to the consumption of contaminated food and feed (Reddy and Raghavender, *et al.,* 2007). In humans, consumption of aflatoxin-contaminated food sometimes leads to severe liver injury and, rarely results in the death. Other symptoms include hemorrhage (bleeding), edema (swelling of body tissue), and changes in metabolism and nutrient absorption, which can lead to malnutrition. Aflatoxicosis is a toxic hepatitis leading to jaundice and, in severe cases, death. Repetitive incidents of this nature have occurred in Kenya (1981, 2001, 2004 and 2005), India, and Malaysia (Shephard, 2004; Lewis *et al*., 2005). AFB_1 has been extensively linked to human primary liver cancer in which it acts synergistically with HBV infection and was classified by the International Agency for Research on Cancer (IARC) as a human carcinogen (Group 1 carcinogen) (IARC, 1993). This combination represents a heavy cancer burden in developing countries.

AFB_1 is metabolized by the liver through the cytochrome P450 enzyme system to the major carcinogenic metabolite AFB_1-8, 9-epoxide (AFBO), or to less mutagenic forms such as AFM_1, Q_1, or P_1 (Crespi and others 1991, Shimada and Guengerich 1989). The exo-form of AFB_1 readily binds to cellular macromolecules including genetic material, for example, proteins and DNA, to form adducts. It is the formation of

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DNA-adducts, such as with N7-guanine, that leads to gene mutations and cancer.

Removal or inactivation of aflatoxin in food and feedstuffs is a major global concern. Aflatoxins can be detoxified from contaminated foods and feeds by physical, chemical and biological methods. Biological detoxification method involves the use of enzymes for the degradation and detoxification of aflatoxin modifying of toxins that led to less toxic products. Studies in this area were significantly increased with the current advances in the field of microbiology, molecular biology and genetic engineering. Various physical and chemical methods have been developed to decrease the Aflatoxins, but these methods have many limitations, such as loss of product nutrition, organoleptic qualities, undesirable health effects, and prohibitive cost of equipment's. These disadvantages encouraged recent emphasis on biological methods of degradation of aflatoxins (Gao *et.al,* 2011).

Teniola *et al*. (2005) studied the ability of cell free extracts of *R.. erythropolis* DSM 14303, *Nocardia corynebacterioides* DSM 12676*, N. corynebacterioides* DSM 20151, and *Mycobacterium fluoranthenivorans* sp. DSM 44556T for aflatoxin degradation. Dramatic reduction of AFB_1 was observed during incubation in the presence of *R. erythropolis* cells (17% residual AFB_1 after 48 h and only 3-6% residual AFB_1 after 72 h).AFB_1 was effectively degraded by extracellular extracts from *R. erythropolis*liquid culture. (Alberts *et al*., 2006). The biodegradation of aflatoxin B_1 (AFB_1) by *Rhodococcus erythropolis* was examined in liquid cultures using thin layer chromatography (TLC), high performance liquid chromatography (HPLC), electro spray mass spectrometry (ESMS) and liquid chromatography mass spectrometry (LCMS).

Ruiyu Zuo *et al. (*2012) carried out a study to examine the ability of combined beneficial microbes to degrade aflatoxin. The optimal proportion of beneficial microbes such as *Lactobacillus casei*, *Bacillus subtilis* and *Pichia anomala* were selected. Wei Zhang *et al.,* (2014) Screened a Strain of *Aspergillus niger* for degradation of Aflatoxin B_1. *Aspergillus niger* could remove 26.3% of aflatoxin B_1 after 48 h of fermentation in nutrient broth (NB).

Hackbart *et al.,* (2014) evaluated the ability of the microorganisms *Rhizopus oryzae* (CCT7560) and *Trichoderma reesei* (QM9414), producers of generally recognized as safe (GRAS) enzymes, to reduce the level of aflatoxins B_1, B_2, G_1, G_2, and M_1.Melvin S. Samuel *et al* (2014) explored the ability of *Pseudomonas putida* to degrade aflatoxin B_1 (AFB_1). The toxigenic strain of *A. flavus* was isolated from sugarcane and used to produce AFB_1 in yeast extract sucrose medium.

MATERIALS AND METHODS

Chemicals &Reagents

All the culture media were purchased from Hi-Media (Mumbai, India).All solvents and reagents were purchased from SRL (Mumbai, India). AFB_1 was obtained from Sigma-Aldrich (Steinheim, Germany) and thin layer chromatography plates from Merck (Silica gel G60 F254, Darmstadt, Germany).

Production and Extraction of Crude AFB_1 from Toxigenic Strain of A.Flavus

The toxigenic strain of *A.flavus* was used for the study, which was earlier reported from our laboratory (Abhishek *et al.,* 2015). Fungal spores were inoculated in 200ml of SMKY medium (Table 1.0) and incubated at room temperature for 22 days to produce AFB_1 (Abhishek *et al.,*2015). After the incubation, the culture media (broth) was filtered using Whatman filter paper. The filtrate was mixed with equal amount of chloroform, shaken well and the solvent part was recovered using separating funnel. This was subjected to evaporation by covering with perforated aluminum foil and kept in dark for 2 days. The residue was collected using 1ml of chloroform, transferred in vial, covered with foil and stored at -20ºC for future requirement.

Table 1 Composition of SMKY mediumpH -5.6±0

Sl.No	Components	Amount(g/L)
1	Sucrose	200
2	$MgSo_4.7H_2O$	0.5
3	KNO_3	0.3
4	Yeast extract	7.0
5	Distilled water	1000 ml

Collection of samples and Isolation of AFB_1 Degrading bacteria

A total of four samples viz., sample IFS1 and IFS2 from groundnut infected and UIFS3, UIFS4 from groundnut uninfected were collected from Gandhi Krishi Vignana Kendra (GKVK), Bengaluru and two different types of oil seeds were collected from K.R. Market, Bengaluru (Table 2.0)

The collected soil samples were subjected forisolation of AFB_1 degrading bacteria following the standard procedure with minor modifications (Ciegler *et al.,* 1966). Briefly, 1g of soil sample serially diluted and dilutions of 10^{-4}, 10^{-5} and 10^{-6} were used for the isolation of AFB_1 degrading bacteria and 100µl of sample was plated on the modified czapek Dox Agar (Table 3.0) to check the viability of bacteria in presence of AFB_1.

Table 2 Different samples collected from various locations for the isolation of AFB_1 Degrading bacteria

Sl.No	Sample code	Sample type	Locations
1	IFS1	Soil	Groundnut infected field, GKVK, Bengaluru.
2	IFS2	Soil	Groundnut infected field, GKVK, Bengaluru.
3	UIFS1	Soil	Groundnut uninfected field, GKVK, Bengaluru.
4	UIFS2	Soil	Groundnut uninfected field, GKVK, Bengaluru.
5	CAS1	Oil seed	K.R.Market, Bengaluru.
6	GN1	Oil seed	K.R.Market, Bengaluru.

Table 3 Composition of Modified Czapek Dox Agar medium

Components	Amount (g/l)
Sucrose	30
$MgSo_4.7H_20$	0.5
KH_2PO_4	3.0
$FeSO_4$	1.0
KCl	0.01
Yeast extract	0.5
Agar	0.05
AFB_1	1.0 mg

The collected oil seed samples were soaked in 100 ml conical flasks containing 20 ml of sterile 0.85% NACl and were kept in shaker incubator for 90min at 350rpm. The resulting suspensions (100µl) were plated on NA medium and incubated

for 72 h at 30ºC and 100µl of sample was plated on the modified czapek Dox Agar (Table 3.0) to check the viability of bacteria in presence of AFB_1.

Antimicrobial activity

Determination of antibacterial assay by disc diffusion method

The 20 ml of sterilized Mueller Hinton Agar was poured into sterile petri plates after solidification, 100 µl of fresh culture of human pathogens gram positive (SA2- *Staphylococcus aureus* ATCC 2593) and gram negative (EC1- Escherichia coli ATCC 25922, ST1- *Salmonella typhi*, KP1- *Klebsiella pneumoniae*) bacteria, all of which are opportunistic human pathogens were swabbed on the respective plates. The discs were kept over the agar plates using sterile forceps at cell supernatants of test bacterial isolates and plates were incubated for 24 hours at 37^0C. After incubation, the diameter of inhibitory zones formed around each disc were measure in millimeter (mm). The readings were taken in three different fixed directions in all 3 replicates and the average value was tabulated.

Determination of antibacterial assay by agar well diffusion method

The 20 ml of sterilized Mueller Hinton Agar (MHA) was poured into sterile petri plates, after solidification, 100µl of fresh culture of human pathogens gram positive (SA2- *Staphylococcus aureus* ATCC 2593) and gram negative (EC1- *Escherichia coli* ATCC 25922, ST1- *Salmonella typhi*, KP1- *Klebsiella pneumoniae*) bacteria, all of which are opportunistic human pathogens were swabbed on the respective plates. The wells were punched over the agar plates using sterile 5mm cork borer and thecell supernatants of test bacterial isolates added to the wells. The plates were incubated for 24 hours at 37^0C. (Mahalingam, 2011) After incubation the diameter of inhibitory zones formed around each well were measured in mm. The readings were taken in three different fixed directions in all 3 replicates and the average value was tabulated (Preethi R, 2010).

Aflatoxin biodegradation designs

Culturing of bacteria in a peptone broth

Overnight cultures of test bacteria were prepared by inoculating each organism in 5 ml of NB and incubated at 37ºC (Gao *et al.,* 2011) The NB without inoculation served as a control. Standard AFB_1 (0.1 µg) was dissolved in 10µl of chloroform in 2mL vial and allowed to evaporate in the dark at room temperature. After complete evaporation., 1mL of bacterial broth culture was added to each of vial and incubated at different intervals of time.

Screening the bacterial strains to study the kinetics of AFB_1 degradation

For the screening of isolated bacterial strain for aflatoxin degradation, studies were conducted according to protocol of Mohsen *et al*., (2012). For the degradation studies, 200µl of pre-inoculum was inoculated in 5ml NB containing 200µl of AFB1.Inoculated cultures were incubated for 3, 6, 12,24 and 48h.The following 3 controls were included:(a)sterile NB (b)NB inoculated with 200µl of cell extract(c) NB inoculated with 100µl of AFB1.All the above-mentioned procedures were done in duplicates. The kinetics of AFB1 degradation by bacterial strain was estimated by measuring bacterial cells absorbance at 600nm.Each experiment was terminated by addition of 5ml chloroform to extract the AFB_1 content.

Reduction of AFB1 from aqueous solution by bacterial cell extract and cell free supernatant fluid of isolated bacterial strain

The effects of bacterial cells and cell free supernatant fluid (extracellular fraction) on reduction of AFB1 were studied according to Tenio *et al*.,(2005). Briefly, the experiment was performed in 2ml Eppendorff tubes in a final volume of 750µl.This involved the addition of 20µl stock solution of AFB1 dissolved in methanol to 730µl of cell free extract. The mixture was incubated in the dark at 30ºc without shaking for 3, 6, 12, 24 and 48 h for the optimal reaction or incubation time studies. Each experiment was terminated by the addition of750µl of HPLC grade chloroform for the extraction of remaining AFB1. All the reactions were done in duplicates.

Enzyme properties

Effects of p^H treatment on AFB1 by bacterial cell free supernatant fluid of isolated bacterial strain

The initial pH value was obtained by adjusting pH to 3.0 and 5.0 with 100 mM citrate acid buffer, and to 7.0, 9.0 and 11.0 by 50 mM sodium phosphate buffer and kept for 24 hours in incubation. Controls were set by adjusting NB medium to different pH values. All experiments were carried out in triplicates. Each experiment was terminated by the addition of 750 µl of chloroform for extraction of residual AFB_1.

Determination of Percentage of degradation of AFB_1

The eluted TLC chromatograms were further analyzed using fluorescence detector for AFB1 quantification. The AFB1 control was considered as 100% and treated AFB1 were compared and percent degradation of AFB1 was analysed accordingly. The percentage of AFB1 degradation was calculated using the following formula

(1-AFB_1 band area in treatment / AFB_1 band area in control) × 100%

Extraction and quantification of Residual $AFB_1$by HPLC

AFB_1 was separated isocratically on Perkins Elmer S- LC-10ATVP, Japan HPLC chromatograph, connected to a reverse phase column PHENOMENEX of particle size 5 µm C18(2) 100 Å, LC Column 250 x 4.6 mm with Shimazu SPD10A uv-vis, Japan fluorescence detector (Perkin-Elmer) and LCI-100 computing integrator. Measurements were made by peak area. The mobile phase was combination of Acetonitrile and orthophosphoric acid in the ratio of 70:30 (Filtered through 0.2 µm Millipore filter) at a flow rate of 1ml/min and detection was observed by fluorescence with excitation at 362nm (λ_{ex}). Quantification of aflatoxin was performed by measuring peak areas at their retention times and comparing them with the relevant standard calibration curve. The identity of AFB_1 was confirmed in all the analyzed treated samples by injecting the sample extracts sequentially and comparing the peak area ratio with the corresponding standard AFB_1 (Younis M.H. *et al*., 2003).

Identification of active AFB1 degrading bacteria

Standard approved protocols were presented for the identification of isolated AFB_1 degrading bacterial strains from all the above-mentioned samples in accordance with Bergey's *Manual of Determinative Bacteriology* (Holt *et al*., 1994). Cell form and size, gram staining, spore formation, motility, colony pigmentation, and production of UV-fluorescent pigments were tested. The strain was also identified by determination of 16S rRNA gene sequences. The genomic DNA of studied bacteria was extracted (Govindarajan *et al*., 2007), and the 16S rRNA was subjected for amplification in Polymerase chain reaction (PCR) using genomic DNA as template and bacterial universal primers.

16S-FP	AGAGTTTGATCMTGGCTCAG
16S-RP	TACGGYTACCTTGTTACGACTT

Statistical analysis

Values were expressed as Mean ± standard error. Analysis of variance (ANOVA) was performed, and the differences between values were tested for significance by Turkeys multiple comparison tests employing the SPSS 20 (IBM, USA) programme. Differences at $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Isolation of AFB1 Degrading Bacteria

Fifteen different several types of bacteria were isolated from different samples, after culturing the isolates in modified Czapek Dox agar medium at 37ºC for 48h. These isolates were collected, and pure cultures were prepared on NA media, which were used for further biodegradation assays. The isolates were able to grow on modified CDA media contain AFB_1, which indicates that they may have the potential to degrade AFB_1 by utilizing it as carbon source.

Antimicrobial activity

Antibacterial activities of cell supernatants were investigated by disc diffusion and agar well diffusion method The aflatoxin degraded organisms *Bacillus, Lactobacillus, Serratia and pseudomonas* were investigated for antibacterial activity against human pathogens *Klebsiella, E.coli* ,*Staphylococcus aureus* and *Pseudomonas*. The maximum zone of inhibition was observed in *Pseudomonas* against *E.coli* followed by bacillus against *Klebsiella* which clearly indicates that the tested human pathogens were showed potent antibacterial activity against *Klebsiella* and *E.coli*.

Table No.4 Determination of antibacterial assay of *test organisms*

	Zone of Inhibition in mm			
Test Organism	***Bacillus Spp.***	***Lactobacillus Spp.***	***Seratia Spp.***	***Pseudomonas Spp.***
S. aureus	12.2±1.247	8.3±0.472	7.3±0.10	6.3±0.20
E.coli	5.3±0.372	17.3±0.37	8.3±0.41	36.2±0.27
Klebsiella pneumoniae	31.6±0.220	18.2±0.300	8.6±0.27	11.6±0.30
Salmonella typhi	25.2±0.35	14.2±0.200	7.0±0.2	5.20±0.27

Values are mean inhibition zone (mm) ± S.D of three replicates

The pathogenic organism *Staphylococcus* aureus and *Salmonella* were showed less zone of inhibition against *Pseudomonas* indicates that antibacterial activity was not effective. So, the tested pathogenic organism *Pseudomonas* is resistant towards *Salmonella* and *Staphylococcus aureus*. (Table 4)

AFB1 degradation assay

Bacterial biodegradation of fifteen isolates in culture medium containing AFB_1 as carbon source was determined using solvent extraction and chromatographic methods by measuring observing the fluorescence under UV (200-400 nm) after different period of incubation. Among fifteen isolates, four isolates showed maximum degradation when they are inoculated into peptone broth containing AFB_1 as a carbon source.

The results revealed that the isolated bacterial strain at the 48 hours of incubation showed maximum degradation of AFB_1, which may be attributed to their ability to utilize AFB1 to a maximum extent as carbon source, where as in other hours of incubation the amount of degradation was less. Culture supernatant of isolate No, IFS2 could degrade 61% AFB1 after 48 hours of incubation compared to 46% and 51% by cell extracts and viable cells, respectively (Fig1). Whereas, culture supernatant of IFS3could degrade 54% AFB1 after 48 hours of incubation and 38% by cell extracts and 42% by viable cells. Cell free culture supernatant was more effective than viable cells and cell extracts (Fig 2).

AFB1 degradation by the culture supernatant of the bacterial isolates shows pH sensitive. In case of IFS2, the highest degradation (54 %) was observed at pH 3 and it decreased gradually as the pH value went up and in case of IFS3, degradation (52 %) at pH 9. This result shows that IFS2 degrade AFB1 better in acidic condition whereas in case of IFS3 degrade better in basic condition. The ability of AFB1 degradation with pH values is typically for enzymatic reactions. Enzymes have an optimal pH range for maximal activities. At pH values outside of the optimum, enzymatic activity decrease due to the ionization of a critical amino acid residue within the catalytic site. The maximal AFB1 degradation by IFS3 was observed at a basic pH (pH 9) and IFS2 at acidic pH (pH 3), indicating the enzyme produced by the isolate had a higher optimal pH compared to enzyme in cell extracts.

The results revealed that the isolated bacterial strain at the 48 hours of incubation showed maximum degradation of AFB_1, which may be attributed to their ability to utilize AFB1 to a maximum extent as carbon source, where as in other hours of incubation the amount of degradation was less. Culture supernatant of isolate No. UIFS5 could degrade 51.3% AFB1 after 48 hours of incubation compared to 39.6% and 32.5% by cell extracts and viable cells, respectively (Fig 3.0). Whereas, culture supernatant of UIFS7could degrade 52.5% AFB1 after 48 hours of incubation and 40.8% by cell extracts and 36.4% by viable cells. Cell free culture supernatant was more effective than viable cells and cell extracts (Fig5.0)

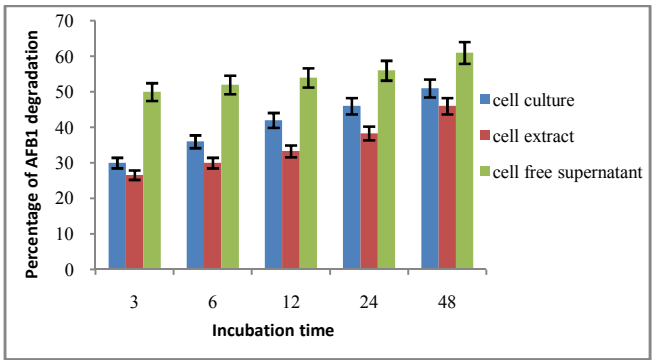


Fig 1 Degradation of AFB1 in the presence of liquid culture, cell extract and cell supernatant of *isolate IFS2.* within different incubation times.

Data given are mean of three replicates ±SE; anlysis of variance (ANOVA) $p < 0.001$

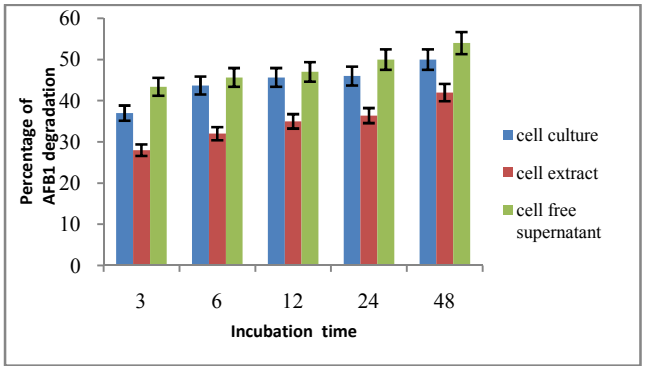


Fig 2 Degradation of AFB1 in the presence of liquid culture, cell extract and cell supernatant of *IFS3.* within different incubation time.

Data given are mean of three replicates ±SE; anlysis of variance (ANOVA) $p < 0.001$

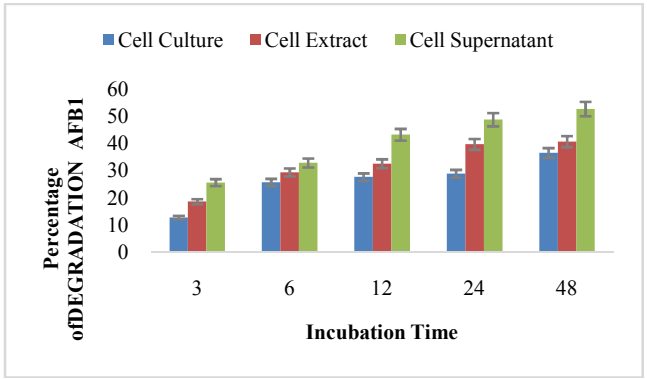


Fig 3 Degradation of AFB_1 in the presence of liquid culture, cell extract and cell supernatant of U*IFS5* different incubation time.

Data given are mean of three replicates ±SE; anlysis of variance (ANOVA) $p < 0.001$

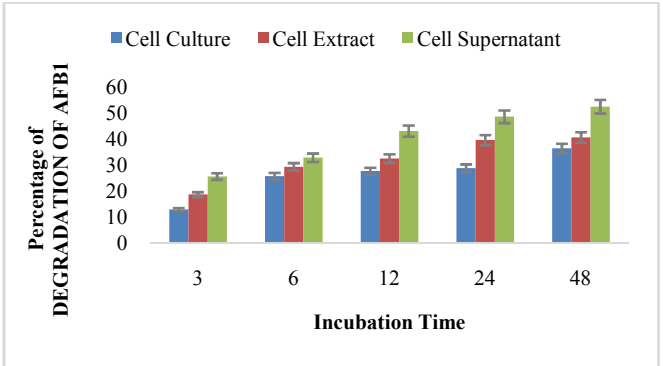


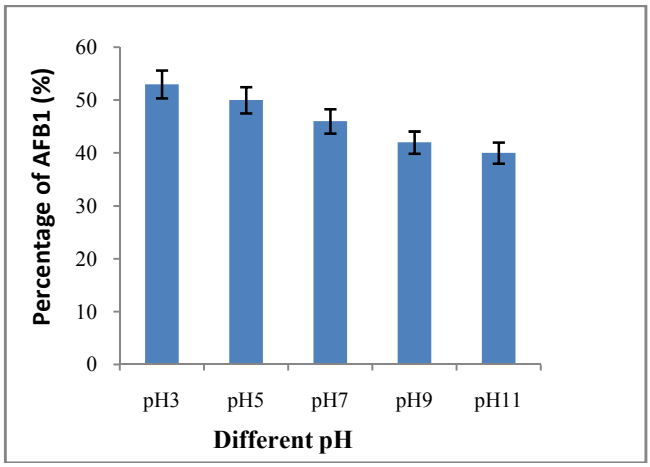
Fig 4: Degradation of AFB_1 in the presence of liquid culture, cell extract and cell supernatant of U*IFS7* different incubation time.

Data given are mean of three replicates ±SE; anlysis of variance (ANOVA) $p < 0.001$

Effect of pH on AFB1 degradation by cell free supernatant of bacterial isolates

The ability of the isolated bacterial strain to degrade Aflatoxin under pH different condition was determined using solvent extraction, chromatographic methods by observing the fluorescence under UV light.

The results reveal that, the AFB1 degradation by the culture supernatant of the bacterial isolates shows pH sensitive (fig 18). In case of IFS2, the highest degradation (54 %) was observed at pH 3 and it decreased gradually as the pH value went up and in case of IFS3, degradation (52 %) at pH 9. This result shows that IFS2 degrade AFB1 better in acidic condition whereas in case of IFS3 degrade better in basic condition. The ability of AFB1 degradation with pH values is typically for enzymatic reactions. Enzymes have an optimal pH range for maximal activities. At pH values outside of the optimum, enzymatic activity decrease due to the ionization of a critical amino acid residue within the catalytic site. The maximal AFB1 degradation by IFS3 and UIFS5 was observed at a basic pH (pH 9) and IFS2 and UIFS7 at acidic pH (pH 3), indicating the enzyme produced by the isolate had a higher optimal pH compared to enzyme in cell extracts (Fig 5 and 6).



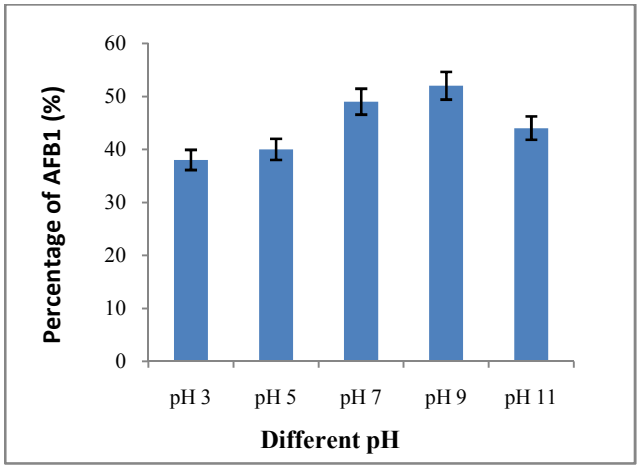


Fig 5 Effect of pH on AFB1 degradation by cell free supernatant of the isolate IFS2 and IFS3.

Data given are mean of three replicates ±SE; anlysis of variance (ANOVA) $p < 0.001$

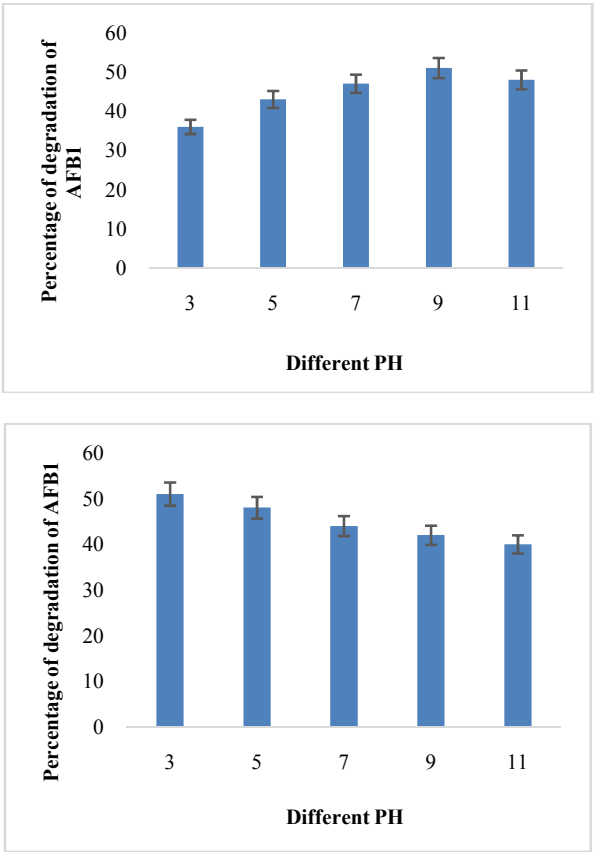


Fig 6 Effect of pH on AFB1 degradation by cell free supernatant of the isolate UIFS5 and UIFS7.

Data given are mean of three replicates ±SE; anlysis of variance (ANOVA) $p < 0.001$

Extraction and quantification of Residual AFB_1 by HPLC

The results of HPLC reviled that there is reduction in the aflatoxin B_1 in the treated samples compared with standard AFB_1 at 48 hrs of incubation by the isolate *Bacillus thurunigensis* and *Seratia sps*. (Fig 7.0 A and B)

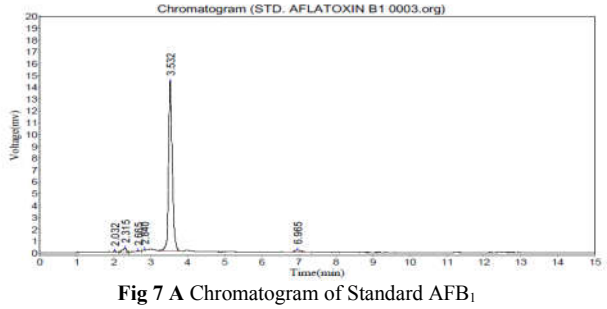
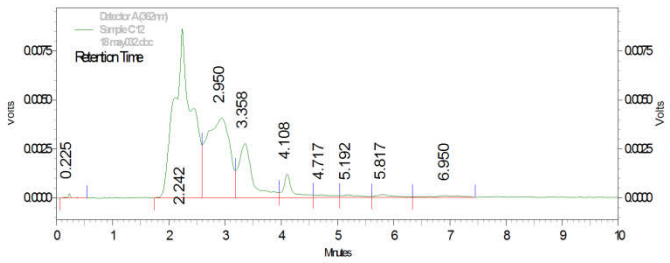
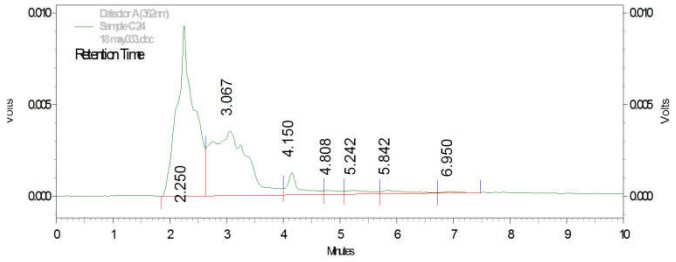


Fig 7 A Chromatogram of Standard AFB_1



A



b

Fig 7 B Chromatogram of treated AFB_1 at 48hrs a) *Bacillus sp* b) *Seratia sp*

Characterization of bacterial isolates

Physiological and biochemical standard tests were performed following the method of Holt *et al 1994*. Briefly, The colony characteristics of the bacterial isolates IFS2, IFS3, UIFS5 and UIFS7 were recorded and results of Gram staining and biochemical assays were recorded (Fig 8.0 &Table 5.0).Based on the efficiency of degradation of AFB_1,theisolate IFS2 among four isolates subjected for characterization by 16S rRNA gene sequencing. The desired sub cultured organism was subjected to genomic DNA isolation and sequencing by colony PCR method &analyzed ABI 3500 XL Genetic Analyzer and the genomic DNA size must be estimated 1.5 Kbp and 16S rRNA gene sequence showed that IFS2 belonged to the strain *B. Thuringiensis* with and the 626bp nucleotide sequence was provided with a GenBank accession number MG407658.

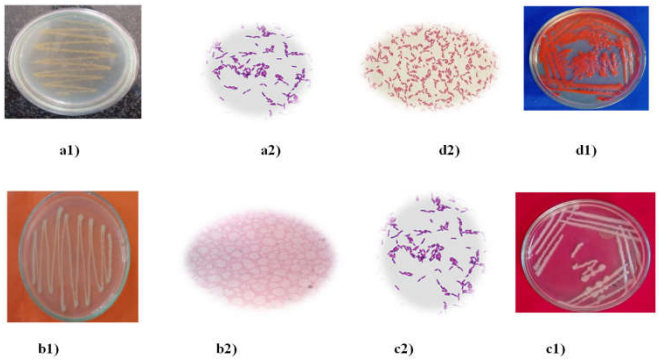


Fig 8 a1 & a2- Pure culture& microscopic View of IFS2; b1 & b2- Pure culture& microscopic View of IFS3; c1 & c2- Pure culture& microscopic View of UIFS5 and d1 & d2- Pure culture& microscopic View of UIFS7.

Table 5 Gram and biochemical characteristics of AFB_1 degrading bacterial strains

BIOCHEMICAL TEST	*IFS2*	*IFS3*	*UIF5*	*UIF7*
Gram Characteristics	+	-	+	-
CATALASE	+	+	+	+
OXIDASE	+	+	-	-
INDOLE	-	-	-	-
MR	-	-	-	-
VP	-	-	-	+
UREASE	+	+	_	+
CITRATE	+	+	-	+
STARCH	-	+	_	+
SPORES	-	-	-	-

'+' Positive response '-' Negative response

CONCLUSION

Aflatoxins are highly toxic polyketide secondary metabolites produced mainly by A. flavus. The human health impact of AFB_1 exposure is widespread in developing countries. It is

known that AFB_1 causes teratogenicity, immunotoxicity, hepatotoxicity and even death in humans and farm animals (Abhishek *et al.,* 2015). Various physical and chemical methods have been implicated to detoxify the aflatoxins, but these methods have many disadvantages such as loss of product nutrition, organoleptic properties, undesirable health effects and prohibitive cost of equipment's. These limitations encouraged recent emphasis on degradation of aflatoxins biologically (Gao *et al.,* 2015).

The present research describes the isolation of bacteria from soil and oil seeds with the ability to detoxify AFB_1. Among a collection of degradative bacteria isolates, the strains IFS2, IFS3, UISF5 and UISF7 were selected based on its ability to utilization of Aflatoxin B_1 (AFB_1) at concentration of (2µg/mL) as a sole carbon source. Thin layer chromatography (TLC) and Spectro-densitometric methods were employed to extract the residual AFB_1concentrations. IFS2 had strong ability to degrade the highly toxic AFB_1. Moreover, the results implied that the activity of aflatoxin B_1 detoxification was mainly noticed in the cell supernatant of IFS2 rather than cell extract and viable cells culture. The cell supernatant was characterized by considerable activity at wide range of temperatures and P^H. The isolate No.*Bacillus Sp*. IFS2 characterized by 16S rRNA sequencing and the results indicated that isolate belongs to Bacillus thuringiensis and the 626bp nucleotide sequence was provided with a GenBank accession number MG407658 and the isolate No. Bacillus Sp. IFS2 supernatant indicate a potential and promising application for detoxification of AFB_1 in the food and feed industry. Biosafety results indicated that AFB_1 detoxification is an enzymatic and extracellular enzyme had the high ability to detoxify. Research was underway to purify the extracellular enzymes and genes responsible for AFB_1 detoxification.

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