بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

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Metagenome Based Analysis of Aflatoxin Degrading Bacteria in Poultry Farms

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Abstract

Aflatoxicosis is a type of poisoning caused due to the consumption of ubiquitous carcinogens called aflatoxins which are produced by toxigenic *Aspergillus* species and contaminate food and agricultural commodities extensively posing major threats to both human health and livestock productivity, especially in the poultry industry. Several decontamination solutions were proposed, but biodegradation methods seemed to be the most attractive solution owing to its specificity and eco-friendliness.

Our study aimed to screen and identify aflatoxin degrading bacteria using both metagenome based methods and traditional methods. Ten bacterial isolates were screened using coumarin as a sole carbon source in media (traditional method), their degradation rates ranged between (25-100%) when tested against AFB1, AFB2, AFG1 & AFG2 using HPLC analysis. Biochemical and molecular identification of these isolates revealed that they belong to four genera; *Bacillus* (6), *Pseudomonas* (2), *Enterococcus* (1) *and Stenotrophomonas* (1) including *Pseudomonas fluorescens* SZ1 that showed the highest degradation rate (~100% to all aflatoxins) after incubation at 37 °C for 72 hrs. in liquid media. While the Metagenome based analysis (microbiome analysis) of 4 environmental samples (2 soil & 2 feed) associated with variable aflatoxin degradation rates revealed that the major taxa usually associated with AF contamination are *Proteobacteria, Firmicutes, Bacteroidetes*, *Actinobacteria* and *Cyanobacteria*. On the genus level, *Bacillus spp* were identified as significantly dominant bacteria in active feed degrading samples while family *Streptophyta* were significantly important in active soil samples. New key microbes usually ignored by conventional culture dependent techniques were present in high AF degrading samples using metagenome based technology, such as *Jeotgaliococcus spp, Halomonus spp*, *lysobacter* and *Streptophyta*. Additionally in our study laccase and lactonase genes for *Bacillus spp* (model) were screened in both culturable isolates and metagenomic samples using primers developed by our team. Insilico analysis and docking of both laccase and lactonase proteins in *Bacillus haynesii* showed promising interactions of both proteins against aflatoxins. Our study suggests biocontrol potential of several different species isolated from poultry farms; *B. haynesii, B. licheniformis, B. tequilensis, B. subtilis, B. amyloliquefaciens, Pseudomonas fluorescens, Enterococcus*

casseliflavus, and *Stenotrophomonas maltophilia* and other important genera weather culturable or not as *Jeotgaliococcus spp*, *Halomonus spp*, *lysobacter* highlighting the importance of metagenomics in screening novel bacteria and catabolic genes that can be used in biotechnology. The results also proposed *Pseudomonas fluorescens* SZ1 as an excellent candidate for bioremediation of aflatoxin in feed matrices while our Insilico analysis revealed that purified products (lactonase & laccase) from *B. haynesii* can be excellent candidates for biotechnology applications in biodegradation of aflatoxins.

Keywords: aflatoxins, *Firmicutes, Proteobacteria*, biodegradation, bioremediation and decontamination, *Bacillus, Pseudomonas*

Dedication

I would like to dedicate this work to my lovely parents and my dear brothers whom without my sky would fall.

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List of abbreviations

Abbreviation	Full name
$(NH4)_2SO_4$	Ammonium sulphate
A°	Angstroms
ADTZ	Aflatoxin-detoxifizyme
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFD	Aflatoxin derivative
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
AFM2	Aflatoxin M2
AFO	Aflatoxin oxidase
AFs	Aflatoxins,
AFT	Aflatoxicol B
AHL	N-acyl-homoserine lactone
Ala	**Alanine**
ALTs	Altertoxins
AME	Alternariol methyl ether
AOH	Alternariol
Asp	Aspartic acid
Asn	Asparagine
ATs	Alternaria toxins

aw	water activity
BLAST	Basic Local Alignment Search Tool
BLASTn	Basic Local Alignment Search Tool for nucleotide
BLASTp	Basic Local Alignment Search Tool for protein
BEA	Beauvericin
Bp	Base pair
bw	Body weight
$CaCl_2.2H_2O$	Calcium chloride dihydrate
CDD	CDD conserved domain database
Chia	Chitinase enzyme
CM	Coumarin media
d	Days
DON	Deoxynivalenol
DSSP	Dialog System for Structured Programming
EAs	Ergot alkaloids
ENNs	Enniatins
EOs	Essential oils
ESpript	ESpript Easy Sequencing in PostScript
EU	European Union
ExPASy	Expert Protein Analysis System
FAO	Food and Agriculture Organization of the United Nations
FBs	Fumonisins
Fe3O4	Ferric oxide
$FeSO_4.7H2O$	Iron sulphate heptahydrate

FISABIO	Foundation for the Promotion of Health and Biomedical Research of the Valencia Region, Spain
FP	Fusaproliferin
g/d	gram/day
Glu	Glutamic acid
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
His	Histidine
HPLC	High pressure liquid chromatography
hrs / h.	Hours
IARC	International Agency for Research on Cancer
Ile	Isoleucine
kcal/mol	kilocalories/mole
KDa	kilo Dalton
KH_2PO_4	Potassium dihydrogen phosphate
KOH	Potassium hydroxide
LAB	Lactic acid bacteria
Lac	laccase enzyme
LD 50	Lethal dose of a compound that kills 50% of population
LDA	Linear discriminant analysis
LEfSe	Linear discriminant analysis Effect Size
Leu	Leucine
Lys	Lysine
mg/kg	Milligram/ kilogram

$MgSO4.7H_2O$	Magnesium sulphate heptahydrate
min.	Minutes
ml	Milliliter
MMN	Montmorillonite nanocomposites
mm	Millimeter
mmol L-1	Milli mole/ Liter
MnP	Manganese peroxidase
MON	Moniliformin
MR	Methyl red
MSM	Minimal Salt Media
MultAlin	Multiple sequence alignment tool
NA	Nutrient agar
$Na_2HPO_4.12H_2O$	Disodium hydrogen phosphate dodecahydrate
NB nutrient broth	Nutrient broth
NCBI	National Center for Biotechnology Information
NHL	N-Homoserine lactone domain
nm	Nanometer
OTs	Ochratoxins
PAH	Polyaromatic hydrocarbons
PAT	Patulin
PCoA	PCoA principle coordinate analysis
PD	Phylogenetic diversity
PDA	Potato dextrose agar
PDB	Protein data bank

Phe	Phenylalanine
phyre2	Protein Homology/AnalogY Recognition Engine
PI	Isoelectric point
Pi-Alkyl	Interaction of pi electric cloud of aromatic group and electron group of any alkyl group
Pi-anion	Interaction of pi electric cloud of aromatic group and electron group of any anion atom
Pi-cation	Interaction of pi electric cloud of aromatic group and electron group of any cation atom
ppb	Part per billion
ppm	Part per million
Pro	Proline
QIIME	Quantitative Insights Into Microbial Ecology
SAVES	Structural Analysis and Verification Server
Ser	Serine
spp.	Species
TAE	Tris-Acetate-EDTA
TCs	Trichothecenes
TeA	Tenuazonic acid
TSI	Triple sugar iron
Tyr	Tyrosine
U/L	Units per liter
UniFrac	Unique fraction
Val	Valine
YASARA	Yet Another Scientific Artificial Reality Application