

**ANTIBACTERIAL ACTIVITIES OF MYCOTOXINS FROM NEWLY
ISOLATED FILAMENTOUS FUNGI**

Praveena YSN and Padmini Palem PC*

Department of Biotechnology, L. Bullayya PG. College, Visakhapatnam, India

*Department of Botany, Andhra University, Visakhapatnam, India.

ABSTRACT : Five different mycotoxins viz., Aflatoxin, Malformin, Penitrem, Patulin and Deoxynivalenone (DON) were extracted from indigenous strains of *Aspergillus niger*, *Aspergillus flavus*, *Penicillium expansum* and *Fusarium graminearum*. Crude mycotoxins were further purified by column chromatography and characterized through TLC with reference to standards. Further antibacterial activities of crude as well as purified mycotoxins were evaluated against selected bacteria. Purified forms were highly inhibitory when compared to crude ones, which were evident through the increased zones of inhibition. The outcome of the present study demonstrates susceptibility of all the bacteria tested with isolated mycotoxins and out of all DON scored maximum inhibitory efficiency.

Key words: Filamentous fungi, Mycotoxins and Antibacterial activity.

INTRODUCTION

Impact of microbial diversity favors probability of identifying microorganisms that produce bioactive compounds, which can be developed into new molecules that fight against many pathogens. Microbial natural products are resources for novel secondary metabolites and filamentous fungi among them remain the most promising source. According to Keller [1] secondary metabolites produced from fungi vary in production, function and specificity to a particular fungus. Krohn [2] described that the natural function of secondary metabolites often is unknown and it is assumed that they play a pivotal role in chemical defense and communication. Gloer [3] found that the biosynthesis of secondary metabolites does not occur randomly but is correlated with ecological factors. Dreyfuss and Chapela [4] made an assumption that certain physical and biological change in natural environment favors the production of a diverse range of secondary metabolites. According to Bandow et al., [5] emergence of multi-drug resistant pathogens was reported to be one of the leading cause of deaths world-wide. Many pathogenic bacteria are becoming resistant to synthetic drugs and hence an alternative strategy is very much needed. Fungi represent an enormous source for natural products with diverse chemical structures and activities and apparently, majority of fungi inhabiting the world have not yet been described. The main focus of present study lies in the investigation of mycotoxins isolated from indigenous strains of filamentous fungi and their inhibitory activity against selected pathogenic bacteria.

MATERIALS AND METHODS

Fungal cultures and production media for mycotoxins

Four filamentous fungi viz, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium expansum* and *Fusarium graminearum* were newly isolated from soil and further evaluated in terms of macro and micro morphological features for confirmation before proceeding to the experimental protocols. Pure cultures were maintained on potato dextrose agar. *A. flavus* was used for production of aflatoxin, *A. niger* for malformin, *P. expansum* for patulin as well as penitrem and *F. graminearum* for DON. Specific production media were used for each mycotoxin viz, corn steep liquor for both aflatoxin and malformin. Potato dextrose for patulin, Czapek Dox yeast extract for penitrem and sucrose peptone for DON. Fungal spore suspensions of concentration 3×10^8 /ml were inoculated to specific media in conical flasks emended with streptomycin to prevent growth of bacteria. The cultures were then incubated for over a period of 7 days in an orbital shaker at 26°C and 150 rpm.

Bacterial cultures

Four test organisms *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus* and *Proteus mirabilis* were procured from Institute of Microbial Technology, Chandigarh, India. Cultures were maintained on nutrient agar slants and were sub cultured in petri dishes prior to testing.

Extraction of crude mycotoxins and purification through column chromatography

Fungal cultures incubated for seven days were subjected to vacuum pump filtration. Further the filtrates were solvent extracted with ethyl acetate and chloroform in ratio 1:1 except for DON where acetonitrile and water in 86:14 ratios were used. After evaporation, the residues were dissolved in DMSO at a concentration of 100µg/ml and purified by column chromatography using silica and specific eluents. For malformin, water and ethyl acetate in ratio 1:1 were used and for aflatoxin, chloroform in 0.7% ethanol was employed. Acetonitrile, chloroform and water in ratios 1:2:1 for patulin, ethylether for DON, methanol and water in ratio 5:1 for penitrem were used.

Characterization of purified mycotoxins by TLC

Thin glass plates were coated with slurry of adsorbent silica and water in ratio 1:2 with 0.25 mm thickness. Purified mycotoxin samples were applied as spots along with standards on the glass plates and placed in a chamber having solvent. TLC was performed till solvent reached top of the plates which were then removed and observed under UV Transilluminator for characterization of purified mycotoxins.

Antibacterial studies

Antibacterial activities of crude as well as purified mycotoxins were evaluated against selected bacteria by agar well diffusion method. 50 µl of the previously prepared mycotoxins were inoculated into 2mm wells made at the centre of petri plates. For each test, three replicates were maintained and in case of controls pure solvent instead of mycotoxins was used. Test as well as control petri plates were incubated at 37 °C for 24 hrs and the zone of inhibition were measured in mm.

RESULTS

TLC analysis aided in characterization of all the mycotoxins isolated. R_f values of both crude and purified mycotoxins were calculated and compared with standards. The values obtained were nearly same when compared to standards and represented high degree of purity (Table 1).

Table – 1 R_f values of crude, purified and standard samples of mycotoxins

Mycotoxins	R _f values of crude samples	R _f values of purified samples	R _f values of standard
Aflatoxin	0.50	0.54	0.56
Malformin	0.54	0.57	0.57
Penitrem	0.70	0.72	0.77
Patulin	0.29	0.30	0.32
DON	0.68	0.70	0.71

Aflatoxin: Antibacterial activities of crude as well as purified forms were evident in all the tested bacteria. Zone of inhibition ranged from 12 mm to 22 mm for crude extract against *E. coli* while purified form showed a range from 16 to 25 mm (Fig.1) *P. Mirabilis* proved to be the most susceptible among bacterial strains tested.

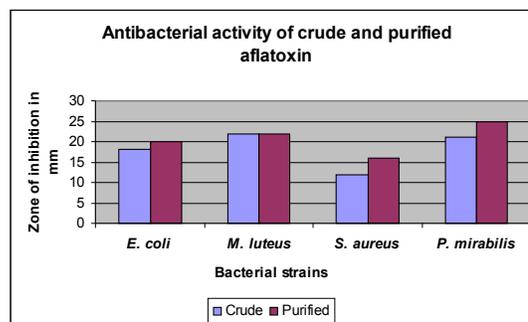


Fig. 1 Antibacterial activities of crude and purified aflatoxin

Malformin: Most susceptible bacterial strain among the tested proved to be *P. mirabilis* which recorded 34 mm zone of inhibition with purified form of malformin (Fig. 2) while crude form was inhibitory towards *S. aureus*. Zones of inhibition in rest of the bacterial strains ranged from 20 mm to 28 mm with crude extract while that of purified ranged from 24 mm to 34 mm.

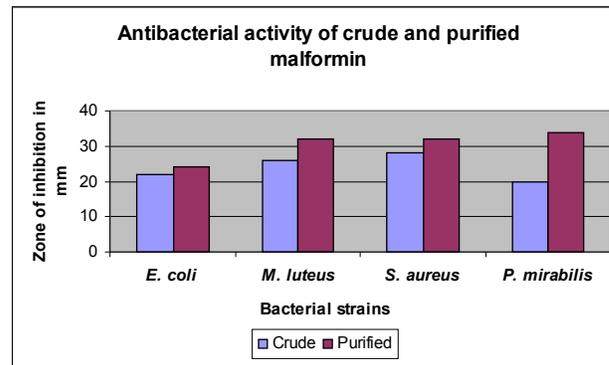


Fig. 2 Antibacterial activities of crude and purified malformin

Penitrem: Antibacterial activity of penitrem was evident in all the tested strains. A Maximum of 35mm zone of inhibition in *S. aureus* and 27mm in *P. mirabilis* by the purified form was recorded (Fig. 3). Results with the crude form ranged from 21 mm to 26 mm in *S. aureus* and *E. coli*.

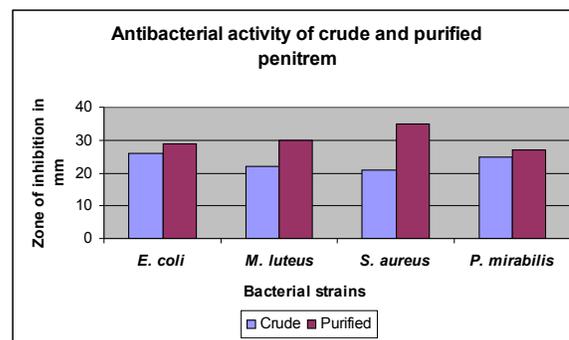


Fig. 3 Antibacterial activities of crude and purified penitrem

Patulin: Sensitivity of bacterial strains towards crude as well as purified forms was prominent. Most susceptible strain was *E. coli* for both crude and purified forms of patulin. Zone of inhibition ranged from 16 to 31 mm in crude and on the other hand for purified patulin, it ranged from 18 to 32 mm in *P. mirabilis* and *E. coli* (Fig. 4).

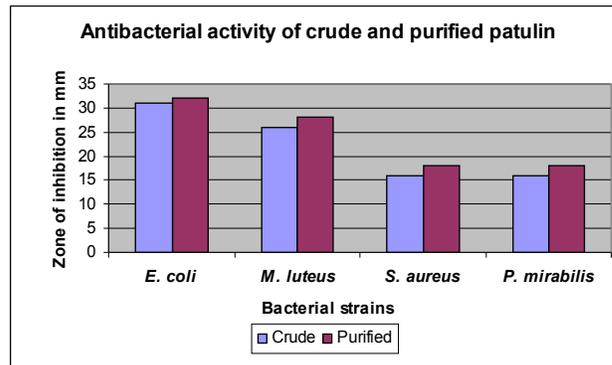


Fig. 4 Antibacterial activities of crude and purified Patulin

DON: Maximum zone of inhibition was observed in *E. coli* in case of both crude and purified forms of DON. Zone of inhibition ranged from 27 to 52 mm in *S. aureus* and *E. coli* with crude extract and for purified form it ranged from 46 to 61 mm in *M. luteus* and *E. coli* (Fig. 5).

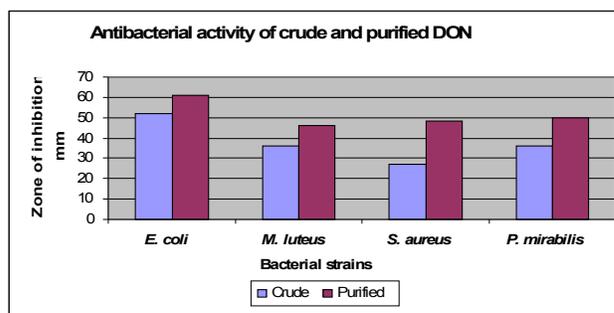


Fig. 5 Antibacterial activities of crude and purified DON

DISCUSSION

A vast number of fungi have been utilized for biotransformation process and many more to be exploited for isolation of potential compounds. According to Demain [6] fungi are well known to show antibacterial, antifungal, larvicidal, molluscicidal, antioxidant and free radical scavenging activities. Findings of the present investigation revealed the inhibitory action of mycotoxins in both crude and purified forms tested against selected strains of bacteria. Bacterial strain *P. mirabilis* was highly susceptible to aflatoxin while, *M. luteus* exhibited same zone of inhibition for both crude as well as purified forms indicating the potential of mycotoxin. In accordance to our findings, Tadashi et al., [7] also reported aflatoxin to be toxic towards *E. coli*, *M. luteus* and *S. aureus* at a concentration of 100 μ l. Crude as well as purified forms of patulin appeared to be inhibitory to *E. coli* and *S. aureus* while *P. mirabilis* proved to be less susceptible. Lee and Röschenthaler [8] assumed that Patulin interferes with DNA and RNA synthesis by inhibiting the incorporation of phosphorous and in later stages it also affects protein synthesis.

In case of malformin, most sensitive among the four bacterial strains observed was *P. mirabilis*. In case of *E. coli* and *S. aureus* not much difference was observed with crude and purified forms. Similar reports were also given earlier by Kobbe et al., [9] and further it was also suggested by Suda and Curtis [10] that malformin interferes with the action of thiol groups which play an important role in auxin metabolism. Penitrem was mainly inhibitory towards *S. aureus* followed by *M. luteus* and *E.coli*. On the other hand DON proved to be toxic towards *E. coli* followed by *P. mirabilis* and *S. aureus* and in contrast to our findings, Harold et al., [11] reported that DON had no inhibitory effects on the growth of bacteria. Antibacterial activities in the present investigation indicate great variation among bacteria as well as mycotoxins tested. All the five mycotoxins in crude as well as purified forms were influential towards bacterial strains tested and of all the mycotoxins, DON proved to be extremely inhibitory. In conclusion, newly isolated filamentous fungi can be exploited for the production of aforementioned mycotoxins which had antibacterial activity against clinically important bacteria. Secondary metabolites represent a large source of compounds endowed with ingenious structures and potent biological activities and according to Siddhardha et al., [12] many of the products currently used for human/ animal therapy, in animal husbandry and in agriculture are produced by microbial fermentation or derived from chemical modifications of a microbial product. Hence the present study suggests that these metabolites and fungal strains can be further utilized for biotechnological applications in medicine and agriculture.

REFERENCES

1. Keller N P, Turner G, Bennet J W. Fungal Secondary Metabolism -From biochemistry to genomics. National Reviews in Microbiology, 2005, 3 (12) : 934-947.
2. Krohn K. 2nd International conference on harmful and beneficial microorganisms in grassland, pastures and turf (ed. Krohn, K.; Paul, V.H.), 1996, 19 (7) : 83-93.
3. Gloer J B. The chemistry of fungal antagonism and defense. Canadian Journal of Botany, 1995, 73, 1265-1274.
4. Dreyfuss M M, Chapela I H. The discovery of natural products with therapeutic potential (ed. Gullo, V.P.); Butterworth-Heinemann, Stoneham, 1994: 49-80.
5. Bandow JE, Brotz H, Leichert LIO, Labischinski H, Hecker M. Proteomic approach to understanding antibiotic action. Antimicrobial Agents and Chemotherapy, 2003, 47: 948-955.
6. Demain A L. Pharmaceutically active secondary metabolites of microorganisms, Applied Microbiology and Biotechnology, 1999, 52, 455-63.
7. Tadashi A Tatsuya I and Yasumasa K. Antimicrobial activity of aflatoxins. Journal of Bacteriology, 1967, 93(1): 59-64.
8. Lee K S and Röschenthaler R J. DNA damaging activity of patulin in *E.coli*. Applied and Environmental Microbiology, 1986, 52(5): 1046-1054.
9. Kobbe B, Cushman M, Wogan G N, And Demain A L. Production and antibacterial activity of malformin C, a toxic metabolite of *Aspergillus niger*. Applied and Environmental Microbiology, 1977: 996-997.
10. Suda S and Curtis R W. Antibiotic properties of malformin. Applied Microbiology, 1966, 14 : 475-476.
11. Harold D M, Qingzong Wu, Cheryl k B. Canadian Journal of Microbiology, 2000, 46(8) : 692-699.
12. Siddhardha B, Prabhakar P, Suryanarayana M U, Venkateswarlu Y. Isolation and Biological Evaluation of Two Bioactive Metabolites from *Aspergillus gorakhpurensis*, Records of Natural Products, 2009, 3(3):161-164.