



Original research article

## Effect of indigenous fungi on ochratoxin A produced by two species of *Penicillium*

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## ABSTRACT

Interaction between indigenous fungal strains in preventing ochratoxin A (OTA) production by *Penicillium verrucosum* and *Penicillium nordicum* was studied in 100 mL of Czapek yeast autolysate (CYA) medium in a 250-mL "U" shaped culture vessel in one end for 3 days. At the end of incubation period, test fungi inoculated and incubated at  $27 \pm 2$  °C for another 14 days to study the inhibition of OTA production was estimated by high performance liquid chromatography (HPLC). Total inhibition of OTA production was recorded with *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ustus*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium proliferatum*, *Penicillium chrysogenum*, *Penicillium expansum* and *Trichoderma viridae*. A significant correlation coefficient ( $r$ ) on growth ( $0.493$ ,  $P \leq 0.0003$ ) and OTA production ( $0.785$ ,  $P \leq 0.0001$ ) was observed between the tested *Penicillium* species and other co-existing fungi. In conclusion, the present investigation revealed that those indigenous fungi are necessary to minimize potential losses to the poultry farmer and toxicological hazards to the consumer as biological control agent in different foods and feeds.

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### 1. Introduction

India is predominately an agrarian country with nearly three fourth of its people depending on agriculture or rural economy. It is apparent that with the stagnant crop productivity, prevention of pre- and post-harvest losses of agricultural products is very important for overcoming the growing food demand. Processing of food grains for safety and quality of processed products is still a challenge for the food industry. Microbes pose a significant threat to food safety, of which mycotoxin contamination is one of the main concerns. Recently poultry and livestock industry has gained prominence in country economy. Among the mycotoxigenic fungi present in poultry feeds, *Penicillium* species and their mycotoxins

are reported to affect feed quality and human health (Bennet and Klich, 2003). Mycotoxins cause a broad range of animal health problems, such as reduction in animal productivity, immunosuppression, damage to vital organs, infertility, and in extreme cases death may occur (Fink-Gremmels and Malekinejad, 2007). Ochratoxin A is one of the most important mycotoxins of worldwide concern for human and animal health. It exhibits a wide range of health effects including nephrotoxicity, mutagenicity, teratogenicity and immunotoxicity (O'Brien and Dietrich, 2005), affects protein synthesis and inhibits ATP production (Xiao et al., 1996). Compounded poultry feeds are frequently contaminated with variety of moulds such as species of *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, *Cladosporium* and *Alternaria* (Magnoli et al., 2007). However, very little information is available on poultry feeds and subsequent hazards in India (Koteswara Rao et al., 2011).

In ancient times, the storage of agricultural products was mostly primitive, but with the advent of technology more scientific methods are developed which prevent mould infestation. A promising strategy to reduce mycotoxin contamination in foods and feeds is to involve the biological interaction between the non-toxicogenic and toxicogenic strains of the same species (Dorner et al., 2003). Meanwhile, several researchers have focused on detoxification of mycotoxins by biotransformation reactions which

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include acetylation, hydrolysis, deamination and decarboxylation (McCormick, 2013). Several fungi, such as species of *Phoma*, *Mucor*, *Rhizopus*, *Alternaria* and *Trichoderma*, are reported to prevent aflatoxin B<sub>1</sub> production by *Aspergillus flavus* (Calistru et al., 1997). However, there are no such studies on the influence of indigenous mycobiota on OTA production in poultry feed by *Penicillium* species.

## 2. Materials and methods

### 2.1. Reagents and standards

Standard OTA (98% TLC), acetonitrile, acetic acid, water HPLC grade Sigma Aldrich (Mumbai, India). Thin-layer chromatography (TLC) plate, oxalic acid, toluene, ethyl acetate, formic acid, o-phosphoric acid, sodium hydroxide and all media chemicals were obtained from Merck (Mumbai, India).

### 2.2. Fungi used in the present study

In all 45 strains of fungi, *Alternaria*, *Aspergillus*, *Cladosporium*, *Chaetomium*, *Dreschlera*, *Fusarium*, *Penicillium*, *Rhizopus*, *Trichoderma*, and ochratoxigenic *Penicillium verrucosum* and *Penicillium nordicum* isolated from poultry feed samples of Andhra Pradesh, India (Koteswara Rao et al., 2011) were used for these studies. The isolated indigenous fungi were screened for their ability to prevent the growth and OTA production by *P. verrucosum* and *P. nordicum*.

### 2.3. Interaction of fungi

Interaction of fungi with other co-existing fungi in a liquid medium was performed according to Rafiyuddin et al. (2001). Briefly, with minor modifications, 7-day-old *P. verrucosum* and *P. nordicum* were inoculated in to CYA broth in a 250 mL “U” shaped culture vessel containing 100 mL of broth in one end and incubated at 27 ± 2 °C. At the end of 3 d incubation period, different test fungi were inoculated with 1 mL of spore suspension at the other end and incubated under same conditions for another 14 d to study the inhibition of OTA production. At the end of 17 d incubation period, culture broth was filtered through Whatman No. 42 filter paper. The culture filtrate was extracted twice with chloroform (1:1) and concentrated by evaporation rotary evaporator, eluted in 500 µL of methanol was used for high performance liquid chromatography analysis.

### 2.4. Chromatographic analysis

Chromatographic analysis of OTA was performed using JASCO-975 (Japan), C-18 isocratic reverse phase column (250 mm × 4.6 mm internal diameter, 5 µm particle size) by injecting 20 µL of sample extract as per our previous report (Koteswara Rao et al., 2013). Significance difference of the data tested by one sample *t* test and coefficient of variation were applied to compare the growth and OTA production using Graph Pad InStat version 5.03 (Graph Pad Software, Inc.)

## 3. Results

### 3.1. Interaction of fungi in a liquid medium

The interaction of *P. verrucosum* and *P. nordicum* with different co-culturing feed-borne fungi revealed that complete inhibition of OTA production by *P. verrucosum* in the presence of *Aspergillus fumigatus*, *A. flavus*, *Aspergillus niger*, *Austus ustus*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium proliferatum*, *Penicillium chrysogenum*, *Penicillium expansum* and *Trichoderma viridae* was

recorded in addition to these species *Fusarium chlamydosporum*, *Penicillium citrinum*, *Penicillium commune*, *Penicillium griseofulvum*, *Penicillium tricolor* and *Rhizopus stolonifer* were responsible for complete inhibition of OTA production by *P. nordicum*. However, least inhibition of OTA production by *P. verrucosum* was recorded in presence of *Fusarium heterosporum* followed by *Penicillium brevicompactum*, *Aspergillus nidulans* and *Aspergillus terreus* in a descending order. The marginal inhibition of OTA production by *P. nordicum* was recorded by interaction of *Cladosporium cladosporoides*, *Chaetomium albocorium*, *Fusarium sporotrichoides*, *Dreschlera halodes*, *Penicillium alli* and *Penicillium caseifulvum*. The rest of the fungi inhibited OTA production to an intermediate level by both species of *Penicillium*. The mean inhibition of OTA production by *P. verrucosum* ranged 0.0 to 22.9 µg/mL with a coefficient variation 105% under the influence of other co-existing fungi. Statistical analysis showed that mean inhibition of OTA production by *P. nordicum* and ranged 0.0 to 19.2 µg/mL with a coefficient variation 112.5% was recorded. A significant correlation coefficient of 0.785 ( $P < 0.0001$ ) was recorded on inhibition of OTA production by both the species of *Penicillium* with other co-existing fungi. The mean inhibition of growth of *P. verrucosum* 4.1 mg/mL in the presence of other fungi ranged between 1.1 and 4.3 mg/mL with a coefficient variation 50.9%. *P. nordicum* recorded mean inhibition of growth, which previously ranged 4.5 mg/mL from 0.9 to 13.1 mg/mL by other co-culturing fungi. A significant correlation coefficient of 0.493 ( $P < 0.0003$ ) was observed on inhibition of growth of both the species of *Penicillium* by other co-existing fungi (Table 1).

## 4. Discussion

Ochratoxin A contamination is common in cereal based foods and feeds in developing countries like India; hence we attempted a method to reduce the exposure of humans and animals to these mycotoxins by minimising their entrance into the food chain. An integrated approach on factors influencing the growth of moulds will provide an effective control of mycotoxin contamination without imposing extreme steps on one factor. In nature foods and feeds harbour a variety of microorganisms which interact with each other both for space and nutrients (Carla et al., 2014). The present investigations are in agreement with Abrunhosa et al. (2002) who also reported that *A. niger* completely inhibited OTA. *A. niger*, *A. fumigatus* and *A. japonicus* hydrolysed OTA and OTB which were further degraded into OTα (Xiao et al., 1996). Several bacteria, protozoa and fungi were able to degrade OTA by reaction of several enzymes, such as carboxypeptidase A, lipases and some commercial proteases (Abrunhosa et al., 2006). Most of OTA degrading microbes were able to remove the phenylalanine moiety from OTA, which leads to the accumulation of a nontoxic version of OTA, i.e., OTα (Yamazaki et al., 1971). *R. stolonifer* inhibited 54% to 82% of growth and 94% to 100% of OTA production (Varga et al., 2005). These authors further reported significant degradation of OTA by *R. homothallicus*, *R. oryzae*, and *R. stolonifer*, which could detoxify OTA in spiked moistened wheat. *A. flavus* co-culturing with *T. viridae* in corn kernels reduced 73% and 100% aflatoxin B<sub>1</sub>, respectively (Ashraf Mostafa et al., 2013).

Wicklow et al. (2005) observed a positive correlation between aflatoxin inhibition and type of interaction of *A. flavus* with other fungi. However, there are certain exceptions such as interaction with *F. culmorum* and *F. proliferatum* that considerably reduced the inhibition of OTA production. *A. niger* inhibited 100% OTA production and 87% of growth by both the species of *Penicillium* under study (Varga et al., 2000). Further, OTA inhibition was recorded in presence of *A. alternata* (72% to 85%), *C. cladosporoides* (87% to 91%) and *T. viridae* (100%) by both the species of *Penicillium* (Cvetnic and

**Table 1**  
Effect of indigenous fungi on ochratoxin A produced by two species of *Penicillium* isolated from poultry feed.<sup>1</sup>

Indigenous fungi	<i>Penicillium verrucosum</i>				<i>Penicillium nordicum</i>			
	Dry wt, mg/mL	Inhibition, %	OTA, µg/mL	Inhibition, %	Dry wt, mg/mL	Inhibition, %	OTA, µg/mL	Inhibition, %
<i>Alternaria alternata</i>	7.65 ± 0.43	46.83	5.04 ± 0.43	78.00	5.86 ± 0.22	55.30	2.83 ± 0.67	85.30
<i>Aspergillus flavipes</i>	6.58 ± 0.62	54.27	3.14 ± 0.29	86.29	6.03 ± 0.30	53.54	4.23 ± 0.12	78.03
<i>Aspergillus fumigatus</i>	2.64 ± 0.50	81.66	0.00 ± 0.00	100	2.19 ± 0.14	83.29	0.00 ± 0.00	100
<i>Aspergillus niger</i>	1.75 ± 0.62	87.83	0.00 ± 0.00	100	1.66 ± 0.49	87.33	0.00 ± 0.00	100
<i>Aspergillus nidulans</i>	3.64 ± 0.48	74.70	5.98 ± 0.63	73.89	2.29 ± 0.12	82.53	3.72 ± 0.49	80.68
<i>Aspergillus flavus</i>	3.14 ± 0.59	78.17	0.00 ± 0.00	100	0.93 ± 0.21	92.90	0.00 ± 0.00	100
<i>Aspergillus japonicus</i>	6.14 ± 0.41	57.33	1.30 ± 0.23	94.32	1.32 ± 0.25	89.93	2.53 ± 0.22	86.86
<i>Aspergillus terreus</i>	2.32 ± 0.38	83.87	5.86 ± 0.23	74.42	2.43 ± 0.26	81.46	3.39 ± 0.14	82.39
<i>Aspergillus ustus</i>	1.54 ± 0.41	89.29	0.00 ± 0.00	100	3.31 ± 0.29	74.75	0.00 ± 0.00	100
<i>Aspergillus versicolor</i>	2.25 ± 0.37	84.36	4.90 ± 0.21	78.61	5.10 ± 0.25	61.09	1.7 ± 0.20	91.17
<i>Cladosporium cladosporioides</i>	3.29 ± 0.40	77.13	5.57 ± 0.28	75.68	2.37 ± 0.33	81.92	8.86 ± 0.21	53.99
<i>Chetomium albocorium</i>	5.54 ± 0.37	61.15	4.98 ± 0.61	78.26	1.34 ± 0.30	89.77	8.15 ± 0.56	57.68
<i>Dreschlera halodes</i>	3.99 ± 0.31	72.27	2.24 ± 0.24	90.22	5.64 ± 0.39	56.97	6.60 ± 0.33	65.73
<i>Fusarium aethiopicum</i>	7.64 ± 0.57	46.90	0.63 ± 0.11	97.25	7.81 ± 0.33	40.42	1.18 ± 0.06	93.87
<i>Fusarium chlamyosporum</i>	5.00 ± 0.70	65.25	1.86 ± 0.27	91.88	5.45 ± 0.14	58.42	0.00 ± 0.00	100
<i>Fusarium culmorum</i>	1.12 ± 0.14	92.21	0.00 ± 0.00	100	4.63 ± 0.19	64.68	0.00 ± 0.00	100
<i>Fusarium equisetii</i>	6.76 ± 0.67	53.02	1.52 ± 0.16	93.36	8.66 ± 0.42	33.94	1.53 ± 0.38	92.05
<i>Fusarium graminearum</i>	5.40 ± 0.34	62.47	0.00 ± 0.00	100	4.37 ± 0.21	66.66	0.00 ± 0.00	100
<i>Fusarium sporotrichioides</i>	3.99 ± 0.55	72.27	3.18 ± 0.25	86.11	2.49 ± 0.35	81.00	8.20 ± 0.35	57.42
<i>Fusarium proliferatum</i>	3.11 ± 0.16	78.38	0.00 ± 0.00	100	3.35 ± 0.08	74.44	0.00 ± 0.00	100
<i>Fusarium heterosporum</i>	4.25 ± 0.64	70.46	8.18 ± 0.55	64.29	5.37 ± 0.23	59.03	5.39 ± 0.19	72.01
<i>Penicillium aethiopicum</i>	5.96 ± 0.25	58.58	4.58 ± 0.31	80.00	7.95 ± 0.34	39.35	3.49 ± 0.07	81.87
<i>Penicillium alli</i>	4.10 ± 0.42	71.50	2.14 ± 0.19	90.65	5.29 ± 0.15	59.64	7.14 ± 0.25	62.92
<i>Penicillium aurantiogriseum</i>	3.32 ± 0.19	76.92	4.45 ± 0.68	80.45	6.21 ± 0.11	52.63	6.30 ± 0.24	67.28
<i>Penicillium brevicompactum</i>	2.20 ± 0.27	84.71	6.31 ± 0.30	72.45	5.26 ± 0.13	59.87	2.76 ± 0.18	85.66
<i>Penicillium camemberti</i>	4.99 ± 0.67	65.32	5.35 ± 0.43	76.64	4.31 ± 0.39	67.12	5.37 ± 0.22	72.11
<i>Penicillium caseifulvum</i>	4.16 ± 0.54	71.09	4.16 ± 0.44	81.84	1.61 ± 0.13	87.71	7.13 ± 0.54	62.98
<i>Penicillium chrysogenum</i>	3.28 ± 0.62	77.20	0.00 ± 0.00	100	3.77 ± 0.41	71.24	0.00 ± 0.00	100
<i>Penicillium citrinum</i>	2.09 ± 0.14	85.47	2.46 ± 0.30	89.26	6.52 ± 0.30	50.26	0.00 ± 0.00	100
<i>Penicillium commune</i>	5.26 ± 0.13	63.44	5.56 ± 0.19	75.73	5.96 ± 0.23	54.53	0.00 ± 0.00	100
<i>Penicillium crustosum</i>	3.32 ± 0.57	76.92	2.36 ± 0.24	89.96	6.99 ± 0.39	46.68	2.80 ± 0.66	85.46
<i>Penicillium digitatum</i>	4.48 ± 0.32	68.86	1.55 ± 0.48	93.23	4.56 ± 0.30	65.21	2.61 ± 0.71	86.44
<i>Penicillium dipodomys</i>	6.06 ± 0.32	57.88	5.32 ± 0.20	76.77	9.14 ± 0.32	69.71	3.50 ± 0.27	81.82
<i>Penicillium discolor</i>	7.07 ± 0.20	50.86	5.33 ± 0.48	76.73	4.27 ± 0.52	67.42	1.64 ± 0.19	91.48
<i>Penicillium expansum</i>	2.12 ± 0.68	85.26	0.00 ± 0.00	100	5.4 ± 0.30	58.81	0.00 ± 0.00	100
<i>Penicillium flavigenum</i>	8.54 ± 0.91	40.65	4.97 ± 0.56	78.30	4.07 ± 0.34	68.95	2.49 ± 0.32	87.07
<i>Penicillium griseofulvum</i>	4.32 ± 0.19	69.97	1.38 ± 0.17	93.97	1.61 ± 0.13	87.71	0.00 ± 0.00	100
<i>Penicillium italicum</i>	2.91 ± 0.22	79.77	5.49 ± 0.18	76.03	2.49 ± 0.24	81.00	5.60 ± 0.30	70.92
<i>Penicillium nalgiovense</i>	5.85 ± 0.24	59.34	4.52 ± 0.31	80.27	2.45 ± 0.35	81.31	6.31 ± 0.21	67.23
<i>Penicillium olsonii</i>	4.16 ± 0.15	71.09	3.64 ± 0.31	84.11	2.28 ± 0.29	82.60	5.11 ± 0.25	73.46
<i>Penicillium rubrum</i>	3.48 ± 0.30	75.81	3.53 ± 0.14	84.59	5.85 ± 0.21	55.37	1.13 ± 0.25	94.13
<i>Penicillium roqueforti</i>	4.38 ± 0.11	69.56	2.37 ± 0.23	89.65	2.46 ± 0.11	81.23	1.73 ± 0.36	91.01
<i>Penicillium tricolor</i>	5.74 ± 0.44	60.11	0.39 ± 0.33	98.29	4.75 ± 0.30	63.76	0.00 ± 0.00	100
<i>Rhizopus stolonifer</i>	2.47 ± 0.13	82.83	1.33 ± 0.19	94.19	6.00 ± 0.28	54.23	0.00 ± 0.00	100
<i>Trichoderma viride</i>	1.26 ± 0.23	91.24	0.00 ± 0.00	100	5.59 ± 0.22	57.35	0.00 ± 0.00	100
Control	14.39 ± 0.90	0.00	22.91 ± 0.52	0.00	13.11 ± 0.70	0.00	19.26 ± 0.59	0.00

OTA = ochratoxin A.

<sup>1</sup> Data are the means ± standard deviation (SD) of 3 replicate experiments statistically significant at  $P < 0.005$ .

Pepeljnjak, 2007). Fungal degradation of mycotoxin by species of *Phoma*, *Rhizopus*, *Aspergillus*, *Candida*, *Trichoderma* and *Mucor* was well documented (Shantha, 1999). Another approach of mycotoxin detoxification is the binding of metabolites (El-Nezami et al., 2002). According to Carla et al. (2014), biotic interactions between indigenous soil borne-fungi of *A. niger* aggregates, *Trichoderma*, *Cladosporium* and *Acremonium* species completely inhibited the OTA accumulation by *Aspergillus carbonarius*. On the other hand, Valero et al. (2006) recorded increased production of OTA by *A. carbonarius* in the presence of *Eurotium amstelodami* or *Penicillium janthinellum*. The growth and OTA production inhibition in mixed cultures may be attributed either due to competition for specific nutrients or production of antimycotic or anti-mycotoxigenic metabolites produced by co-existing fungi (Shantha et al., 1990). Amal and Soher (2014) excellently reviewed the detrimental effects of mycotoxins strategies and reduced the growth of mycotoxigenic fungi and also to decontamination and/or detoxification in of foods and feeds.

## 5. Conclusion

From the present investigations, it can be concluded that these fungi are potential to use in commercial application of bio-control agents.

## Conflict of interest

We declare that we have no conflict of interest.

## Ethical statement

This article does not contain any studies with human or animal subjects performed by any of the authors.

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