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**Original Article**

# *Candida parapsilosis* as a Potent Biocontrol Agent against Growth and Aflatoxin Production by *Aspergillus* Species

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**Abstract**

**Background:** Aflatoxin contamination of food and feed stuff is a serious health problem and significant economic concerns. In the present study, the inhibitory effect of *Candida parapsilosis* IP1698 on mycelial growth and aflatoxin production in aflatoxigenic strains *of Aspergillus* species was investigated.

**Methods:** Mycelial growth inhibitions of nine strains of aflatoxigenic and non-aflatoxigenic *Aspergillus* species in the presence of *C. parapsilosis* investigated by pour plate technique at different pH, temperature and time of incubation. Reduction of aflatoxin was evaluated in co-cultured fungi in yeast extract sucrose broth after seven days of incubation using HPLC method. The data were analyzed by SPSS 11.5.

**Results:** The presence of the *C. parapsilosis* at different pH did not affect significantly the growth rate of *Aspergillus* isolates. On the other hand, temperature and time of incubation showed to be significantly effective when compared to controls without *C. parapsilosis* ($P \leq 0.05$). In aflatoxigenic strains, minimum percentage of reductions in total aflatoxin and $B_1$, $B_2$, $G_1$, $G_2$ fractions were 92.98, 92.54, 77.48, 54.54 and 72.22 and maximum percentage of reductions were 99.59, not detectable, 94.42, and not detectable in both $G_1$ and $G_2$, respectively.

**Conclusion:** *C. parapsilosis* might employ as a good biocontrol agent against growth and aflatoxin production by aflatoxigenic *Aspergillus* species

**Keywords:** Biocontrol, *Candida parapsilosis*, Detoxification, Aflatoxin $B_1$, HPLC

## Introduction

Aflatoxins (AFTs) are difuranocoumarin derivatives produced via polyketide pathway by many strains of *Aspergillus flavus*, *A. parasiticus*, *A. bombycis*, *A. ochraceus*, *A. nomius* and *A. pseudotamari*. Among them *A. flavus* is not only a medically important agent, but also a common contaminant of many important agricultural products (1, 2). AFTs are potential threats to human and animal health through consumption of AFTs contaminated food and feed stuff. They are considered as potent hepatotoxic and hepatocarcinogenic agent and can adversely affect on cell mediated immune response, causing reduction of phagocytosis and depression of complement and interferon production (3).

The International Agency for Research on Cancer (IARC), classifies naturally occurring aflatoxins and aflatoxin $B_1$ ($AFB_1$) as group 1 carcinogens for human (4). The aflatoxins are food-borne mycotoxins likely to be of greatest impact in Africa and other tropical developing countries. Aflatoxins attract worldwide attention because of the significant economic losses related to their effect on human and animal health (5, 6). Several studies were carried out to diminish the contamination of human and animal foods through chemical, physical, and biological methods that convert the toxins into less harmful materials with less mutagenic effects (7). It is well - known that many microbial agents have antifungal activity against *Aspergillus* section *flavi* (8, 9). It is reported that a number of *Bacillus*, *Pseudomonas*, *Ralstonia* and *Burkholderia* strains could completely inhibit the growth of *A. flavus* (10). Some species of saprophytic yeast such as *Candida krusei*, *Pichia anomala* (11), *P. guilliermondii* (12), *Kluyveromyce* spp. (13) and moulds such as *A. oryzae, A. niger,* and *Rhizopus oryzae* (14,15) have been shown inhibitory effects on the growth of *Aspergillus flavus* and/ or its aflatoxin production in vitro (11,16). Strains of lactic acid bacteria, *Rhodococcus erythropolis* and *Mycobacterium fluorantheniorans* sp. Nov DSM44556T and some strains of *Saccharomyces cerevisiae* showed to have probiotic activity (17-19).

The objective of this study was to evaluate the inhibitory effect of *C. parapsilosis* on mycelial growth and AFTs production of aflatoxigenic *Aspergillus* strains.

## Materials and Methods

### Chemicals and instrumental analyses

The mixture standard solutions of $AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$, purchased from Sigma (St. Louis, MO, USA). Chloroform, acetonitrile, methanol, sabouraud glucose agar (SGA) obtained from Merck (Darmstadt, Germany). Other chemicals and solvents were of analytical grade or HPLC grade. Deionized water was purified by the Milli-Q-Plus ultrapure water system. LCTech (GmbH, Germany) supplied Immunoaffinity column (Afla clean). HPLC apparatus consisted of a Smartline HPLC pump 1000, a PDA detector 2800 and a degasser 5000, all from Knauer (Berlin, Germany). The data were acquired and processed by means of Chrom Gate software (version 3.3.1) from Knauer (Berlin, Germany). Detection performed by fluorescence with excitation and emission wavelengths of 365 and 418 nm (20). Chromatographic separation was achieved on a Lichrospher 100 RP & EC C8 reverse phase column (C8, 25×0.46 cm i.d., 5 µm particle size) from Teknokroma (Barcelona, Spain). 0.45 µm PTFE Membrane Filters were purchased from Schleicher & Schull Germany.

### Microorganism cultivation

Seven isolates of *A. flavus* (three aflatoxigenic and four non- aflatoxigenic) from Iranian patients (IP) with sinusitis, one *A. parasiticus* ATCC15517, one *A. soja* IMI191303 (International Mycological Institute, UK) as positive and negative controls and one isolate of *C. parapsilosis* IP1698 were also included to the present study. This late strain was only one of the 24 clinical isolates of *C. parapsilosis* tested against toxigenic and non- toxigenic *Aspergillus* spp. and presented inhibitory effect on growth and aflatoxin production. All clinical isolates previously identified by standard mycological methods (21). Aaflatoxigenicity of the *Aspergillus* isolates evaluated qualitatively and quantitatively by HPLC method. All isolates were stored in sterile distilled water at room temperature until used in the experimental studies. The *C. parapsilosis* was grown on sabouraud glucose agar (SGA) for 48 h. The number of viable cells estimated by standard plate count method and adjusted to $1\times10^6$ cells/ml. *Aspergillus* strains induced to sporulate on SGA plates at 28 °C for five days. The spore suspension harvested by sterile water/tween 20, 0.05% and vigorous agitation and followed by filtration through sterile whatman paper No. 1 to remove mycelial debris. The densities of the conidia was determined with a Neubauer counting chamber and adjusted to $1\times10^6$ conidia/ml. Conidial viability was confirmed by standard plate count method on SGA (22).

### Growth studies

Primarily 0.5 ml of a suspension containing $1\times10^6$ CFU/ml of *C. parapsilosis* was added to each 10 mm diameter plates with 20 ml of molten SGA (45 ˚C) at pH values of 6.5, 7.4 and 8.0. The plates inoculated in the center with 20 µl of $1\times10^6$ conidial suspension of each strain of *Aspergillus* (22). All plates were incubated at 25, 30, 35 ˚C for 24 and 48 h, respectively. After this period of incubation, the diameter of colony surface of the test organism compared with that of control. All experiments performed in duplicate and repeated twice. The mean diameters of colonies considered for evaluation.

### AFTs production, extractions and clean up and analyses

One ml of $1\times10^6$/ml cells spore suspension of each *Aspergillus* spp. was added to 500 ml sterile 2% yeast extract and 15% sucrose broth (YES) pH 6.5. A volume of 1 ml yeast suspension containing 1–$1.2\times10^6$/ml cells added for treatment of the sample. For maximum AFTs production, all cultures incubated on a rotary shaker 100 rpm at 25 ˚C for seven days (Stuart Co, UK). The contents then harvested through filter and the culture mat dried in an oven at 70 ˚C.

Samples were analyzed using HPLC method (the AOAC official method 999.07 as the same as ISIRI 6872, national standard, and 2004) with some minor modifications (23). A volume of 10 ml chloroform added to 500 mg derided mass cell and agitated vigorously. After 15 minutes, the supernatant was removed and evaporated with $N_2$ under safety cabinet and re-constituted in10 ml phosphate buffer saline pH 7.2 (PBS), and passed through a membrane filter (0.45 µm pore size). Then the filtrate passed through the Immunoaffinity Afla clean column (AFC) at a flow rate of 1 drop/second. The column washed with 10 ml de ionized water. Finally AFTs was eluted using methanol through two following steps, 1 ml methanol was applied on the column and after 5 minutes was allowed to pass by gravity, 1 ml additional methanol was poured on the column after a minute and elute was later collected and 50 µl injected to HPLC. Water/methanol/acetonitrile (60:30:10, v/v/v) were used as the mobile phase with a flow rate of 1.0 ml/min at 25 °C. AFTs reported in mass dried cells of *Aspergillus* spp. with ng/g unit. The retention time was 20 min. A five- point calibration curve drawn for different types of AFTs including $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ to compare and find linear correlation. All tests carried out at least two times for each sample.

### Statistic analysis

The data were analyzed by SPSS 11.5 and four way analysis of variance (ANOVA) followed by Scheffe test was used to distinguish significant differences between before and after treatments. The differences with $P\leq 0.05$ were considered as significant.

## Results

### Effect of C. parapsilosis isolates on growth rates of Aspergillus spp.

Results indicated that presence of the *C. parapsilosis* at different pH did not diminish significantly the growth rate of *Aspergillus* isolates. On the other hand, temperature and time of incubation showed to be significantly effective when compared to controls without *C. parapsilosis* ($P\leq0.05$). Furthermore, growth inhibition of aflatoxigenic and non-aflatoxigenic *Aspergillus* spp. was to be insignificant at tested pH, temperature and time of incubation. Results of maximum and minimum growth condition for test and control isolates summarized in Table 1.

### Effect of C. parapsilosis isolates on AFTs production

An interaction between four aflatoxigenic strains of *Aspergillus* [*A. flavus* IP65938, *A. parasiticus* ATCC15517, *A. flavus* IP53, *A. flavus* IP6641] with selected *Candida parapsilosis* IP1698 in reduction of aflatoxin was observed (Table 2).

**Table 1**: Inhibitory effect of *Candida parapsilosis* IP1698 on mycelial growth of nine *Aspergillus* spp. at various temperature, time and pH

| ***Aspergillus*** spp. | | | 25 ˚C | | 30 ˚C | | 35 ˚C | |
|---|---|---|---|---|---|---|---|---|
| | **pH** | **Time (h)** | **Test Ø mm** | **Control Ø mm** | **Test Ø mm** | **Control Ø mm** | **Test Ø mm** | **Control Ø mm** |
| 1) *A. soja* (IMI191303) [-] | 6.5 | 24 | 0 | 11 | 0 | 13 | 0 | 18 |
| | | 48 | 0 | 17 | 0 | 14 | 8 | 20 |
| | 7.4 | 24 | 0 | 11 | 0 | 13 | 4 | 18 |
| | | 48 | 5 | 17 | 0 | 14 | 8 | 20 |
| | 8.0 | 24 | 0 | 11 | 0 | 13 | 4 | 18 |
| | | 48 | 0 | 17 | 0 | 14 | 8 | 20 |
| 2) *A. flavus* (IP9573) [-] | 6.5 | 24 | 0 | 10 | 0 | 10 | 0 | 20 |
| | | 48 | 2 | 16 | 0 | 14 | 7 | 22 |
| | 7.4 | 24 | 0 | 10 | 0 | 10 | 0 | 20 |
| | | 48 | 0 | 16 | 0 | 14 | 8 | 22 |
| | 8.0 | 24 | 0 | 10 | 0 | 10 | 0 | 20 |
| | | 48 | 0 | 16 | 0 | 14 | 12 | 22 |
| 3) *A flavus* (IP489) [-] | 6.5 | 24 | 0 | 11 | 0 | 11 | 2 | 21 |
| | | 48 | 0 | 14 | 0 | 12 | 2 | 23 |
| | 7.4 | 24 | 0 | 11 | 0 | 11 | 2 | 21 |
| | | 48 | 0 | 14 | 0 | 12 | 2 | 23 |
| | 8.0 | 24 | 0 | 11 | 0 | 11 | 0 | 21 |
| | | 48 | 2 | 14 | 0 | 12 | 8 | 23 |
| 4) *A. flavus* (IP6586) [-] | 6.5 | 24 | 0 | 12 | 0 | 10 | 0 | 20 |
| | | 48 | 1 | 13 | 0 | 11 | 4 | 20 |
| | 7.4 | 24 | 0 | 12 | 0 | 10 | 0 | 20 |
| | | 48 | 2 | 13 | 0 | 11 | 4 | 20 |
| | 8.0 | 24 | 0 | 12 | 0 | 10 | 0 | 20 |
| | | 48 | 0 | 13 | 0 | 11 | 4 | 20 |
| 5) *A. flavus* (IP65483) [-] | 6.5 | 24 | 0 | 12 | 0 | 12 | 4 | 27 |
| | | 48 | 2 | 13 | 0 | 12 | 4 | 27 |
| | 7.4 | 24 | 0 | 12 | 0 | 12 | 4 | 27 |
| | | 48 | 2 | 13 | 0 | 12 | 4 | 27 |
| | 8.0 | 24 | 0 | 12 | 0 | 12 | 4 | 27 |
| | | 48 | 2 | 13 | 0 | 12 | 8 | 27 |
| 6) *A. flavus* (IP65938) [+] | 6.5 | 24 | 0 | 8 | 0 | 9 | 2 | 22 |
| | | 48 | 0 | 14 | 0 | 14 | 2 | 24 |
| | 7.4 | 24 | 0 | 8 | 0 | 9 | 2 | 22 |
| | | 48 | 0 | 14 | 0 | 14 | 2 | 24 |
| | 8.0 | 24 | 0 | 8 | 0 | 9 | 2 | 22 |
| | | 48 | 0 | 14 | 0 | 14 | 4 | 24 |
| 7) *A. parasiticus* (ATCC15517) [+] | 6.5 | 24 | 0 | 15 | 0 | 14 | 8 | 24 |
| | | 48 | 3 | 16 | 0 | 15 | 8 | 25 |
| | 7.4 | 24 | 0 | 15 | 0 | 14 | 8 | 24 |
| | | 48 | 4 | 16 | 0 | 15 | 8 | 25 |

**Table 1:** Cond…

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 8.0 | 24 | 0 | 15 | 0 | 14 | 8 | 24 |
| | | 48 | 4 | 16 | 0 | 15 | 8 | 25 |
| 8) *A. flavus* | 6.5 | 24 | 0 | 14 | 2 | 14 | 10 | 18 |
| (IP53) [+] | | 48 | 0 | 18 | 6 | 19 | 10 | 19 |
| | 7.4 | 24 | 0 | 14 | 2 | 14 | 10 | 18 |
| | | 48 | 0 | 18 | 6 | 19 | 13 | 19 |
| | 8.0 | 24 | 0 | 14 | 2 | 14 | 10 | 18 |
| | | 48 | 0 | 18 | 2 | 19 | 13 | 19 |
| 9) *A. flavus* | 6.5 | 24 | 0 | 15 | 0 | 10 | 3 | 22 |
| (IP6641) [+] | | 48 | 1 | 17 | 0 | 12 | 3 | 22 |
| | 7.4 | 24 | 0 | 15 | 0 | 10 | 3 | 22 |
| | | 48 | 2 | 17 | 0 | 12 | 4 | 22 |
| | 8.0 | 24 | 0 | 15 | 0 | 10 | 3 | 22 |
| | | 48 | 2 | 17 | 0 | 12 | 3 | 22 |

[-]: Non-aflatoxigenic strain, [+]: Aflatoxigenic strain, IP: Iranian patients, Ø: colony diameter millimeter, Time: hours

**Table 2:** Amount of aflatoxin produced by *Aspergillus* spp. before and after treatment with *C. parapsilosis* IP1698

| Aflatoxigenic isolates | Before treatment | | | | | After treatment | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $G_2$ | $G_1$ | $B_2$ | $B_1$ | Total | $G_2$ | $G_1$ | $B_2$ | $B_1$ | Total |
| *A. flavus* (IP53) | 0.18* | 3.30* | 0.74* | 52.08* | 56.31* | 0.05* (72.22)** | 0.03* (99.09)** | 0.11* (85.13)** | 0.03* (99.94)** | 0.23* (99.59)** |
| *A. parasiticus* (ATCC15517) | 14.74* | 2.16* | 9.95* | 9.79* | 36.64* | ND | ND | 2.24* (77.48) ** | ND | 2.25* (93.82) ** |
| *A. flavus* (IP65938) | 0.06* | 0.11* | 0.61* | 24.66* | 25.42* | 0.01* (83.33)** | 0.05* (54.54)** | 0.06* (90.16)** | 0.27* (98.90)** | 0.39* (98.46)** |
| *A. flavus* (IP6641) | 0.40* | 0.68* | 11.83* | 31.25* | 43.76* | 0.08* (80.00)** | ND | 0.66* (94.42)** | 2.33* (92.54)** | 3.07* (92.98)** |

ND: Not detectable, IP: Iranian patients, *: ng/g, **: % of reduction.

## Discussion

In this study, *C. parapsilosis* IP1698 shows significant reduction on the mycelial growth of nine *Aspergillus* isolates in 24 and 48 h. This isolate also exhibits inhibitory effect on AFTs production by aflatoxigenic strains of *Aspergillus* in comparison with control experiment. These results are comparable with the study of La Penna and co-workers in which *Kluyveromyces* sp. isolate decreased mycelial growth and aflatoxin $B_1$ production by aflatoxicogenic *Aspergillus* strains (13). Maximum and minimum conditions for inhabitation growth varied in different strains (Table 3).

The present study also showed that there was insignificant difference between pH and mycelia growth rate in all isolates. Thermal incubation at 25–35, 30–35 ˚C showed to be significantly effective on growth rates of test and control groups ($P \leq 0.05$). Furthermore antifungal effects of , *C. parapsilosis* on *Aspergillus* spp. at 25–30 ˚C were significant only in isolates No. 5, 6, 7 and 8 ($P \leq 0.05$). There was insignificant difference between growth conditions in aflatoxigenic and non-aflatoxigenic strains. Total AFT and $AFB_1$ decreased more than 90% in all aflatoxigenic strains and the best reduction was observed in *Aspergillus flavus* IP53, 99.59 and 99.94%, respectively.

**Table 3:** Maximum and minimum condition for inhibition of mycelia growth in test and control *Aspergillus* isolates

| ***Aspergillus* spp.** | **Maximum mean of colony diameter/mm** | **Minimum mean of colony diameter/ mm** |
|---|---|---|
| 1) *A. soja* (IMI191303)[-] | pH 6.5/35 ˚**C**/24 h (18) | pH 6.5/25 ˚**C**/24 h (11)<br>pH 7.4/25 ˚**C**/24 h (11)<br>pH 8.0/25 ˚**C**/24 h (11) |
| 2) *A. flavus* (IP9573)[-] | pH 6.5/35 ˚**C**/24 h (20)<br>pH 7.4/35 ˚**C**/24 h (20)<br>pH 8.0/35 ˚**C**/24 h (20) | pH 6.5/25 ˚**C**/24 h (10)<br>pH 6.5/30 ˚**C**/24 h (10)<br>pH 7.4/25 ˚**C**/24 h (10)<br>pH 7.4/30 ˚**C**/24 h (10)<br>pH 8.0/25 ˚**C**/24 h (10)<br>pH 8.0/30 ˚**C**/24 h (10)<br>pH 8.0/35 ˚**C**/48 h (10) |
| 3) *A. flavus* (IP489)[-] | pH 6.5/35 ˚**C**/48 h (21)<br>pH 7.4/35 ˚**C**/48 h (21)<br>pH 8.0/35 ˚**C**/24 h (21) | pH 6.5/25 ˚**C**/24 h (11)<br>pH 6.5/30 ˚**C**/24 h (11)<br>pH 7.4/25 ˚**C**/24 h (11)<br>pH 7.4/30 ˚**C**/24 h (11)<br>pH 8.0/25 ˚**C**/24 h (11)<br>pH 8.0/30 ˚**C**/24 h (11) |
| 4) *A. flavus* (IP6586)[-] | pH 6.5/35 ˚**C**/24 h (20)<br>pH 7.4/35 ˚**C**/24 h (20)<br>pH 8.0/35 ˚**C**/24 h (20) | pH 6.5/30 ˚**C**/24 h (10)<br>pH 7.4/30 ˚**C**/24 h (10)<br>pH 8.0/30 ˚**C**/24 h (10) |
| 5) *A. flavus* (IP65483)[-] | pH 6.5/35 ˚**C**/24 h (23)<br>pH 6.5/35 ˚**C**/48 h (23)<br>pH 7.4/35 ˚**C**/24 h (23)<br>pH 7.4/35 ˚**C**/48 h (23)<br>pH 8.0/35 ˚**C**/24 h (23) | pH 6.5/25 ˚**C**/48 h (11)<br>pH 7.4/25 ˚**C**/48 h (11)<br>pH 8.0/25 ˚**C**/48 h (11) |
| 6) *A. flavus* (IP65938)[+] | pH 6.5/35 ˚**C**/48 h (22)<br>pH 7.4/35 ˚**C**/48 h (22) | pH 6.5/25 ˚**C**/24 h (8)<br>pH 7.4/25 ˚**C**/24 h (8)<br>pH 8.0/25 ˚**C**/24 h (8) |
| 7) *A. parasiticus* (ATCC15517)[+] | pH 6.5/35 ˚**C**/48 h (17)<br>pH 7.4/35 ˚**C**/48 h (17)<br>pH 8.0/35 ˚**C**/48 h (17) | pH 7.4/25 ˚**C**/48 h (12)<br>pH 8.0/25 ˚**C**/48 h (12) |
| 8) *A. flavus* (IP53)[+] | pH 6.5/25 ˚**C**/48 h (18)<br>pH 7.4/25 ˚**C**/48 h (18)<br>pH 8.0/25 ˚**C**/48 h (18) | pH 7.4/35 ˚**C**/48 h (6)<br>pH 8.0/35 ˚**C**/48 h (6) |
| 9) *A. flavus* (IP6641)[+] | pH 6.5/35 ˚**C**/24 h (19)<br>pH 6.5/35 ˚**C**/48 h (19)<br>pH 7.4/35 ˚**C**/24 h (19)<br>pH 8.0/35 ˚**C**/24 h (19)<br>pH 8.0/35 ˚**C**/48 h (19) | pH 6.5/30 ˚**C**/24 h (10)<br>pH 7.4/30 ˚**C**/24 h (10)<br>pH 8.0/30 ˚**C**/24 h (10) |

[-]: Non-aflatoxigenic strain, [+]: Aflatoxigenic strain, IP: Iranian patients

Biodegradation of aflatoxins, using microorganisms or enzymes, is one of the well-known strategies for removal of aflatoxins in food and feedstuffs. It is reported that some fungi such as *Trichoderma viride, Paecilomyces lilacinus, Penicillium griseofulvin, Saccharomyces cerevisiae, Candida utilis, Penicillium urticae, Rhizopus nigricans*, and *Mucor rouxii* were able to reduce the total concentration of AFT by 67.2,

53.9, 52.4, 52, 51.7, 44, 38.2 and 35.4%, respectively (24). It has already been shown that several fungi including *Phoma* spp., *Mucor* spp., *Trichoderma* spp., *Rhizopus* spp., and *Alternaria* spp. inhibited the synthesis of $AFB_1$ more than 90% (25). The present study indicated that not only $AFB_1$, but also $AFB_2$, $AFG_1$, $AFG_2$ and total AFT total diminished when treated with *C. parapsilosis*. $AFB_1$ production by *A. flavus* also can be inhibited by *Fusarium proliferatum* after both fungi were co-cultured in optimal growth conditions (26). *Trichoderma* spp. produced volatile compounds that may play an inhibitory role on colony growth and morphology of *A. flavus* and *F. moniliforme* (27).

Biodegradation of harmful compounds actively considered promising choice, since they are specific and environmentally friendly to decrease or remove the possible contaminations of aflatoxins in food and feed stuffs (28, 29). Our study shows that *C. parapsilosis* is able to remove AFTs from liquid (YES) medium as a new report. *Pichia anomala* can produce ethyl acetate (30) which could diminish or inhibit mould growth (31). Specific enzymes that are capable of degrading aflatoxins reported from microbial systems. A new aflatoxin degradation enzyme also was detected and purified from *Pleurotus ostreatus* (32). Fluorescence proportions detailed that the specific enzyme cleaved the lactone ring of aflatoxin. $AFB_1$ was metabolized with *Dactylium dendroides* NRRL2575, *Mucor alterans* NRRL3358, *Mucor griseo-cyanus* NRRL3359, *Absidia repens* NRRL1336, and *Helminthosporium sativum* NRRL3356 (33). Three mechanisms proposed in biological control with antagonists' fungi: a) parasitism, deriving nutrients from the host; b) competition, for space and nutrients; and c) antibiosis, production of an inhibitory metabolite or antibiotic (34). Competition for space or nutrients often recommended as mode of action of biological control against pathogen agents. We have found that the *C. parapsilosis* IP1698 is capable of reducing both growth and aflatoxin production at wide range of pH and temperature. Although the mechanisms of aflatoxin removal are still unknown, however binding aflatoxin molecules onto the cell wall components may be an explanation for this hypothesis. Other investigators showed the addition of cell walls of *Saccharomyces cerevisiae* into feed could lead to reduction in toxic effect of aflatoxin in broilers as well (35, 36). Different fungi have different physiology habitation and may be explained the variation between the other researches. Considering the effect of the interactions between microorganisms under different ecological situation may be useful to know the nature and extent of fungal growth in the stored grain ecology. This may have essential implications for biological control with yeast strains. Cell surface hydrophobicity may play an important role in the binding and cell wall polysaccharides are the most important part responsible for this binding of to toxins (37).

In conclusion, the potent activity of *C. parapsilosis* indicates that, this yeast has an important role in the biodegradation of AFTs. Thus, the present approach to anti-aflatoxin efficacy of *C. parapsilosis* warrants further investigation to make clear the mode of action on toxicity prior to common application.

## Ethical Consideration

All Ethical issues (such as informed consent, conflict of interest, plagiarism, misconduct, co-authorship, double submission, etc) considered carefully.

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