Original Article

The antagonistic effects of *Candida parapsilosis* on the growth of *Fusarium* species and fumonisin production

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Abstract

Background and Purpose: *Fusarium* species are avid producers of secondary toxic and carcinogenic metabolites such as fumonisin. Contamination of food and feed products with fumonisin can be hazardous to the health of humans and animals and may lead to agricultural loss. Accordingly, in this study, we aimed to evaluate the effects of *Candida parapsilosis* on the growth and fumonisin production of *Fusarium* species.

Materials and Methods: Mycelial growth rate 26 *Fusarium* isolates, including *F. verticillioides* (n=6), *F. proliferatum* (n=18), *F. solani* (n=1), and *F. oxysporum* (n=1), in the presence of 42°C. *parapsilosis* strains was investigated by pour-plate method. The decline in fumonisin production was measured in co-cultured fungi in coarsely ground maize after four weeks of incubation in the dark at 22°C, using ELISA technique. For data analysis, paired t-test was performed, using SPSS version 20.

Results: The mycelial growth and fumonisin production of *Fusarium* isolates significantly decreased in the presence of *C. parapsilosis* in comparison with the control cultures ($P<0.05$). The percentage of mycelial growth inhibition ranged from 56.36% to 74.54%. The minimum and maximum decline in total fumonisin production was 12% and 78%, respectively. *F. oxysporum* and *F. solani* were found to be minor fumonisin producers among the studied *Fusarium* species. On the other hand, a decline was reported in the growth of *Fusarium* species and fumonisin production in the presence of *C. parapsilosis.*

Conclusion: *C. parapsilosis* showed notable inhibitory activities against *Fusarium* isolates. Therefore, this fungal species could be considered as a biocontrol agent against the growth and fumonisin production of toxigenic *Fusarium* species in the future.

Keywords: Biocontrol, *Candida*, ELISA, Fumonisins, *Fusarium*, Mycotoxin

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Introduction

Mycotoxins have been long recognized as a global problem for human and animal health. The threat only increases as the demand for available food supplies rises in response to world population growth [1]. Contamination of staple food grains by fungi has been reported in various regions, particularly in developing countries. This sort of contamination can happen at all stages of plant cultivation, i.e., pre-harvest, harvest, and storage stages.

The safety and nutritional quality of foodstuffs are often reduced by fungal toxins, which are metabolites produced by several fungal species, colonizing staple agricultural products and crops exported from developed and developing countries. Mold and mycotoxin contamination may be detected at any point of the supply chain. The climate of tropical and subtropical countries provides ideal conditions for mycotoxin production, which needs to be controlled by post-harvest processes, adequate equipments, and sound management practices [2].

Fusarium species constitute a group of fungi with a worldwide distribution. They are recognized as a common cause of contamination in various grains and a possible source of different mycotoxins, such as fumonisin, zearalenone, and trichothecene [3, 4]. Fumonisins are highly toxic carcinogenic metabolites, which are usually formed in plants, mainly infected with *F. verticillioides* or *F. proliferatum.* These metabolites are commonly found in maize or other agricultural products prior to harvest.

Food and feed stuff contamination with fumonisin is notable for the serious public health hazards and the associated economic burden [5-8]. This mycotoxin, which is structurally similar to

sphingosine, interferes with the metabolism of sphingolipids and leads to cell apoptosis [9]. In the literature, leukoencephalomalacia, porcine pulmonary edema, hepatotoxicity, and hepatocarcinogenicity have been reported as the consequences of intoxication by fumonisin in horses, pigs, and rats, respectively [10-13].

Today, fumonisin B1 is known as a risk factor for high rates of human esophageal cancer in South Africa and China [5-7, 14]. The same probably applies to Iran, as high rates of natural contamination with *Fusarium* strains have been reported in maize, wheat, and rice by Ghiasian et al. [15], Mohammadi-Gholami et al. [16], and Alizadeh et al. [8] in areas with major cultivation and high risk of esophageal cancer.

Use of biocontrol agents is an efficient and cost-effective strategy to control and decrease toxicity by fumonisin [17]. The inhibitory properties of different fungal and bacterial organisms on the growth and mycotoxin production of various toxigenic fungal species have been reported in several studies. In this regard, Turkel et al. [18] introduced *Metschnikowia pulcherrima* UM15 as a highly effective yeast against various *Fusarium*, *Aspergillus*, and *Penicillium* species. Similarly, Rocha et al. [19] showed the major suppressive effect of *Bacillus thuringiensis* on *F. verticillioides* growth and fumonisin production.

The antagonistic activities of *M. pulcherrima* strains against *Candida tropicalis* and *C. albicans* were reported by Csutak and colleagues [20]. Furthermore, the efficient inhibitory activities of *Saccharomyces cerevisiae* RC008 and RC016 strains against the growth and mycotoxin production of *A. carbonarius* and *F. graminearum* were reported by Armando and colleagues [17].

Niknejad et al. used *C. parapsilosis* as a biocontrol agent against the growth and aflatoxigenicity of *Aspergillus* species [21]. Considering the high toxigenicity of *Fusarium* isolates from different regions of Iran, as reported by Mohammad-Gholami et al. [16], control of these toxigenic fungi should be taken into account.

To the best of our knowledge, no study has been conducted on the role of *C. parapsilosis* as a biocontrol agent in decreasing fumonisin production and growth of *Fusarium* isolates. Accordingly, in this study, we aimed to evaluate the effects of *C. parapsilosis* on the growth and fumonisin production of *Fusarium* isolates.

Materials and Methods

Fungal isolates

A total of 42 clinical *C. parapsilosis* isolates and 26 clinical and environmental *Fusarium* isolates were evaluated in this study. All isolates were obtained from the culture bank at the medical mycology laboratory of School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. All fungal strains were stored in sterile distilled water. The working cultures were prepared from distilled water stocks after being transferred to Sabouraud Glucose Agar (SGA; Merck, Germany).

Preparation of yeast and conidial suspensions

Cultures of *C. parapsilosis* on SGA were incubated at 30°C for 48 h and were used to prepare a suspension of yeast cells with a density of 10^6 cells/ml. Spore production by *Fusarium* isolates was induced on SGA plates, incubated at 28°C for 48 h. The spores were harvested in sterile water, containing 0.05% Tween 20, followed by vigorous agitation. Then, the mycelial debris was removed by filtration through sterile Whatman No. 1 paper. Conidial density was adjusted to 10^6 cells/ml, using a haemocytometer slide. The standard plate count method was used on SGA to confirm yeast and conidial viability [21].

Effects of C. parapsilosis on the growth of Fusarium isolates and fumonisin production

In order to determine the inhibitory effects of *C. parapsilosis* on the growth of *Fusarium* isolates, 0.5 ml of each yeast suspension (10^6 cells/ml) was added to each plate (10 mm in diameter), containing 20 ml of molten SGA at 45°C. After solidification, the plates were centrally inoculated with 20 µl of conidial suspension of each *Fusarium* strain. After seven days of incubation at room temperature, colony diameters were measured using calipers.

The growing diameter of cultures, containing *Fusarium* and *C. parapsilosis* isolates, was compared with the control cultures (free of *C. parapsilosis*). For each colony, the two diameters measured at the orthogonal position were averaged to determine the mean diameter for each colony. All experiments were carried out with two separate replicate plates per treatment. The mean diameter of colonies was considered to be indicative of evolution. Afterwards, *C. parapsilosis* isolate with the most significant inhibitory effect on growth was selected by evaluating its inhibitory effect on fumonisin production.

Fusarium isolates were prepared for mycotoxin production on autoclaved, ground maize, according to mycotoxin protocols with minor modifications [15]. Then, 20 g of coarsely ground maize was

moistened with 20 ml of distilled water in 10 mm diameter plates and autoclaved at 121°C for 20 min over two consecutive days. Briefly, 1 ml of *C. parapsilosis* IPC24A inoculum (10^6 cells/ml) was cultured in the prepared maize medium. Afterwards, 1 ml of each *Fusarium* isolate suspension was inoculated in the center of the plates. The suspension was incubated in the dark at 22°C for four weeks; all tests were carried out in triplicate.

Fumonisin extraction and analysis by ELISA protocol

Fumonisin was extracted from 5 g of each maize medium, using 70% methanol and filtration through Whatman No 1 paper. Quantitative ELISA technique for the analysis of fumonisin was performed after extract dilution, using sterile distilled water, according to the manufacturer's instructions in Ridascreen Fumonisin Kit (R-Biopharm, Germany). Considering the high concentration of toxins, further dilutions of the filtrate, i.e., 1:500 and 1:1000, were prepared prior to the assay, using distilled water. The results were multiplied by the dilution coefficient.

Statistical analysis

For statistical analysis, paired t-test was performed, using SPSS version 20. P-value less than 0.05 was considered statistically significant.

Results

The statistical analysis of the results revealed a significant difference in colony diameter and fumonisin production between co-cultured *Fusarium* and *C. parapsilosis* isolates and the control cultures (*Fusarium* isolates alone) ($P<0.05$). The mean colony diameter of the control cultures and co-cultured isolates was 56.7 mm and 19.8 mm, respectively (Figure 1).

Overall, *C. parapsilosis* strains were able to decrease the growth of all *Fusarium* isolates. However, *C. parapsilosis* IPC24A isolated from infected nails was found to be the most effective strain, decreasing the colony diameter of *Fusarium* isolates; therefore, this strain was used throughout the study. In the present study, the difference between decreased growth and fumonisin production among the studied *Fusarium* species was insignificant. The detailed results on colony diameter and amount of fumonisin production by *Fusarium* isolates are presented in Table 1.

Discussion

Cereal crops such as maize and wheat constitute an important part of human food and animal feed. It is estimated that 25% of food crops are contaminated with mycotoxins, produced by toxigenic fungal contaminants [22]. According to the Food and Drug Administration (FDA) of USA, the maximum acceptable level of total fumonisin is 2-4 ppm in corn products consumed by humans [23].

According to the International Agency for Research on Cancer, fumonisin is probably carcinogenic to humans; consequently, it is categorized in group 2B of carcinogens [8, 24-27]. Considering the toxic and potential carcinogenic properties of this mycotoxin, it is necessary to apply cost-effective and technically feasible methods to decrease or remove this hazardous compound [28].

Biological control of phytopathogens using microorganisms has been known as an effective method [17]. Yeasts are among the most efficient microorganisms, used as biocontrol agents. The inhibition of growth and mycotoxin production is generally attributed to the competition between other microorganisms and toxigenic filamentous fungi for nutritional, space, and other requirements [17].

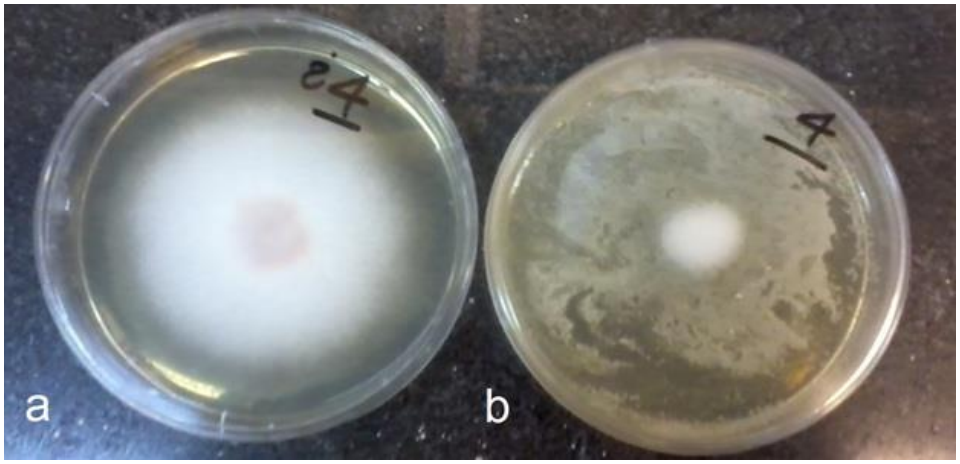

Figure 1. The inhibitory effects of *Candida parapsilosis* on the growth rate of *Fusarium proliferatum* after seven days of incubation at room temperature; a) *Fusarium proliferatum*, b) *Fusarium proliferatum* in the presence of *Candida parapsilosis*

Table 1. The mean colony diameter and amount of fumonisin produced by *Fusarium* species before and after treatment with *Candida parapsilosis* IPC24A

Isolate	Species	Mean colony diameter (mm) before treatment	Colony diameter (mm) after treatment (reduction%)	Fumonisins (ppm) before treatment	Fumonisins (ppm) after treatment (reduction%)
1M	*F. proliferatum*	60	19 (68.33)	310	200 (35.5)
2M	*F. proliferatum*	57	22 (61.4)	200	120 (40)
3M	*F. proliferatum*	55	24 (56.36)	2000	1750 (12.5)
4M	*F. proliferatum*	55	18 (67.3)	2000	1600 (20)
5M	*F. proliferatum*	60	14 (76.7)	220	148 (32.72)
6M	*F. verticillioides*	58	19 (67.24)	2000	1670 (16.5)
7M	*F. verticillioides*	60	23 (61.7)	1450	900 (37.93)
8M	*F. proliferatum*	55	22 (60)	2100	1270 (39.52)
9M	*F. proliferatum*	55	17 (69)	370	296 (20)
10W	*F. proliferatum*	57	22 (61.4)	0.2	0.14 (30)
11M	*F. proliferatum*	60	23 (61.7)	2160	1620 (25)
12M	*F. proliferatum*	54	16 (40.4)	600	365 (39.16)
13M	*F. proliferatum*	55	16 (70.9)	1800	1404 (22)
14W	*F. proliferatum*	52	15 (71.1)	140	100 (28.57)
15W	*F. proliferatum*	55	21 (61.82)	970	737 (24)
16W	*F. proliferatum*	55	22 (60)	2000	260 (87)
17W	*F. proliferatum*	55	14 (74.54)	130	79 (39.23)
18M	*F. verticillioides*	53	15 (71.7)	200	123 (38.5)
19M	*F. verticillioides*	60	23 (61.7)	2000	1760 (12)
20M	*F. verticillioides*	58	21 (63.8)	2970	1200 (59.6)
21C	*F. verticillioides*	55	16 (70.9)	970	580 (40.2)
22C	*F. proliferatum*	60	18 (70)	130	96 (26.15)
23C	*F. proliferatum*	58	18 (68.9)	730	475 (34.93)
24C	*F. proliferatum*	54	23 (57.4)	2100	1650 (21.42)
25C	*F. solani*	58	20 (65.5)	130	90 (30.76)
26C	*F. oxysporum*	60	20 (66.7)	140	85 (39.3)

M: Maize, W: Wheat, C: Clinical, mm: Millimeter, ppm: Parts per million

In a study conducted by Armando et al., *Saccharomyces cerevisiae* was able to inhibit the growth of *A. carbonarius* and *F. graminearum* and prevent the production of ochratoxin A, zearalenone, and deoxynivalenol [17]. This ability of *S. cerevisiae* is speculated to be related to the strain-dependent property of mycotoxin absorption [29]. Therefore, fungi by absorbing mycotoxin lead to the reduction or removal of hazardous compounds. The beta-glucan from the cell wall of *S. cerevisiae* is a probable involved compound in toxin absorption [21].

On the other hand, Csutak et al. reported competition for iron as the major mechanism of antagonistic action in *M. pulcherrima* strains against *C. tropicalis* and *C. albicans* [20]. Matic et al. [30] in a previous study investigated the efficacy of *M. pulcherrima*, *Pichia guilliermondii*, and *P. anomala* as biocontrols against *F. fujikuroi*. They proposed β-1,3-glucanase, fungicides or fungistatic compounds (such as ethanol and ethyl acetate), and hydrolase enzyme secretion as effective mechanisms of *M. pulcherrima*, *P. anomala*, and *P. guilliermondii*, respectively. Based on these findings, the active role of *C. parapsilosis* in our study might be due to one or a combination of these mechanisms.

In the present study, all *Fusarium* isolates from wheat and maize, as well as the clinical isolates, were fumonisin producers. Although *F. oxysporum* is recognized as a non-fumonisin producer species [31], our results revealed fumonisin production by this *Fusarium* species. The co-occurrence of other *Fusarium* toxins including nivalenol, zearalenone, and deoxynivalenol (DON) with other DON derivatives might be a contributing factor, owing to the relative cross-reactivity.

On the other hand, Rheeder et al. introduced *F. oxysporum* (in section *Elegans*) as a fumonisin-producing species [32]. In the present study, *C. parapsilosis* exhibited inhibitory effects against all *Fusarium* isolates. The growth rate and fumonisin production were significantly lower in co-cultured isolates in comparison with *C. parapsilosis*-free cultures. In addition, Niknejad et al. [21] reported the antagonistic effects of *C. parapsilosis* on mycelia growth and aflatoxin production by *Aspergillus* species.

Bacon et al. reported the antagonistic effects of *Trichoderma* species on the growth and fumonisin production of *F. moniliforme* in comparison with the control cultures [33]. Furthermore, the role of *P.*

anomala in *Penicillium verrucosum* as an inhibitor of ochratoxin a production has been suggested in the literature [17]. *A. flavus*, *A. niger*, and *A. ochraceus* have been shown to have the ability to reduce fumonisin production by *Fusarium* species and destroy fumonisin [34]. Similar to the present results, the efficacy of some fungal species as biocontrol agents was confirmed in the mentioned study.

Almost all detoxification methods have some limitations. An ideal approach should be cost-effective, practical, and free of side-effects for humans and animals; therefore, combined application of different methods may be required. Also, unfavorable conditions for mycotoxin production should be considered in the storage period.

Conclusion

The antagonistic properties of *C. parapsilosis* strains are of great significance. Considering the opportunistic and pathogenic nature of this fungus, further research is required on the extracted fractions, effective components, and genes of this fungus for future use as efficient biocontrols against the growth and mycotoxin production of *Fusarium* species. In the present study, the ability of *Fusarium* isolates from different species and sources (particularly clinical isolates) to produce fumonisin was noteworthy.

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Authors' contributions

F.Z. designed and supervised the study. R.K. provided environmental *Fusarium* isolates. P.K., R.D.G., and M.S. provided administrative, technical, and scientific counseling, respectively. B.F. performed all the experiments in the study, and S.M. drafted the manuscript. The final version of the manuscript was revised by F.Z. and S.M.

Conflicts of interest

None.

Financial disclosure

There were no financial interests related to the materials of the manuscript.

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