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Full Length Research Paper

Four strains of yeasts: as effective biocontrol agents against both growth and mycotoxins formation by selected 11 toxigenic fungi

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Spoilage and poisoning of foods by fungi is a major problem, especially in developing countries. The need thus arises for natural preservatives that could be used for semi processed and processed foods. One of these possibilities is the use of yeast strains to control mycotoxigenic fungi. Four yeast strains (*Candida krusei* AUMC 8161, *Pichia anomala* AUMC 2674, *Pichia guilliermondii* AUMC 2663 and *Saccharomyces cerevisiae* AUMC 3875) were selected as a biocontrol agents against both growth and mycotoxins production by different 11 toxigenic fungal isolates; five local isolates from different Egyptian food sources and six isolates obtained from CBS (Central Bureau voor Schimmel cultures, Holland). *Candida krusei* AUMC 8161 completely inhibited the growth and toxin formation of all the 11 tested toxigenic isolates. *Pichia anomala* AUMC 2674 completely inhibited the growth and toxin production by 6 fungal isolates and strongly reduced the growth as well as toxin formation by the other tested toxigenic fungi. *Pichia guilliermondii* AUMC 2663 strongly reduced the growth and toxin production by the 11 toxigenic fungi. *Saccharomyces cerevisiae* AUMC 3875 completely inhibited the growth of 5 fungal isolates and strongly reduced the growth of the others.

Keywords: toxigenic fungi, mycotoxins, antagonistic yeasts.

INTRODUCTION

Many fungi producing mycotoxins are frequent contaminants of foodstuffs and, when conditions are favorable for growth, they grow and produce mycotoxins. Thus it is obvious that if the growth of toxigenic fungi can be prevented subsequent contamination with mycotoxins will also be prevented. The use of many of the available physical and chemical methods for preserving foods from contamination with toxigenic fungi and their toxins is restricted due to problems concerning safety issues, possible losses in the nutritional quality of treated foods and coupled with limited efficacy and cost implications (**Köhl *et al*., 2011**). However, in most countries, chemical and physical preservation are not permitted in foods. The need thus arises for natural preservatives that could be used for semi-processed and processed foods. Currently the global trend is turned to safer and eco-friendly

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alternative approaches (Mari *et al*., 2007; Sharma *et al*., 2009). It has been reported that antagonistic microorganisms or their antimicrobial metabolites have some potential as natural bio-preservatives to control undesirable fungi.

Natural yeasts have been efficacious as biological control agents (Fan and Tian, 2000). Yeasts possess many properties that make them useful for control purposes. Yeasts generally do not produce allergenic spores or mycotoxins as many mycelial fungi do, or antibiotic metabolites likely to be produced by bacterial antagonists (Droby and Chalutz, 1994). Yeasts have simple nutritional requirements and are able to colonize dry surfaces for long periods of time, as well as withstand many pesticides used in the postharvest environment (El-Tarabily and Sivasithamparam, 2006). In addition, yeasts can grow rapidly on inexpensive substrates in fermenters and are therefore easy to produce in large quantities (Druvefors, 2004).

Antagonistic yeasts were shown to reduce the growth of filamentous spoilage moulds both *in vitro* and *in vivo* (McGuire, 1994; Petersson and Schnürer, 1995). The antagonistic yeast *Pichia anomala*, for example, has been shown to reduce *in vitro* the fungal biomass of *Penicillium roquefortii* and *Aspergillus candidus* (Petersson and Schürer, 1995). Many authors have reported the use of yeasts as biocontrols of phytopathogenic filamentous fungi (Montesinos *et al*. 2002; Reyes *et al*. 2004; Coelho *et al*. 2007). Thus, efforts to elucidate antagonistic interactions between yeast and other microorganisms in order to further biological control of phytopathogens are important (Korres *et al*., 2011). So, the present investigation was aimed to evaluate the potential of four yeast strains for bio-control of the fungal growth and toxin production by 11 toxigenic fungi.

MATERIALS AND METHODS

Selection of toxigenic fungi

A total of 11 toxigenic fungal isolates were selected for studying the bio-control activities of four yeast strains on their growth and toxins formation. The toxigenic selected fungal isolates were 5 isolates from different food sources in Sohag Governorate, Egypt and recorded as highly toxin producers (local isolates) named: *Aspergillus flavus* 30 (Aflatoxin B_1, B_2, G_1 and G_2 producer), *A. ochraceus* 76 (Ochratoxins A, B), *Aspergillus nidulans* 69 (Sterigmatocystin), *Penicillium digitatum* 131 (Patulin) and *Alternaria alternata* 5 (Alternariol). The other six highly toxigenic fungal isolates were purchased from CBS (Centraal Bureau voor Schimmelcultures), Fungal Biodiversity Center of Holland and used as a standard isolates. These isolates were *Aspergillus parasiticus* CBS 571.65 (Aflatoxin B_1, B_2, G_1 and G_2), *A. ochraceus* CBS 589.68 (Ochratoxin A), *Penicillium griseofulvum* CBS 589.68 (Patulin), *P. scabrosum* CBS 530.97 (Fumagillin), *Fusarium equiseti* CBS 406.86 (Zearalenone) and *Phaeosphaeria nodorum* CBS 438.87 (Alternariol).

Selection of yeast strains

The yeast strains selected as potential bio-control agents were obtained from Assuit University Mycological Center (AUMC), Egypt. These strains: *Candida krusei* AUMC 8161, *Pichia anomala* AUMC 2674, *Pichia guilliermondii* AUMC 2663 and *Saccharomyces cerevisiae* AUMC 3875.

Testing the effect of yeast strains on growth of toxigenic fungal isolates and their toxins formation

Potato - dextrose liquid medium was used. Erlenmeyer flasks of 250 ml capacity were used. Each flask contained 50 ml medium. The flasks were sterilized at 121 ^{o}C for 20 minutes and inoculated after cooling with the 2 ml of propagated inoculum of one of selected antagonistic yeasts + 2ml of the toxigenic fungal inoculum suspension. At the same time other flasks were inoculated with the toxigenic fungi only served as a control. The cultures were incubated at 28 ± 2 ^{o}C as static cultivation for 10 days. At the end of incubation period, the visible growth rate of each flask was recorded and compared with the control. Then the content of each flask (medium + toxigenic fungus + antagonistic yeast) were homogenized for five minutes in a high speed blender (16000 rpm) with 100 ml chloroform. The extraction procedure was repeated three times. The combined chloroform extracts were washed with equal volume of distilled water, dried over anhydrous sodium sulphate, filtered then concentrated to near dryness. The antagonistic effect of yeast strains on toxins formation was determined for each toxigenic fungal isolates under study as previously described by Korres *et al*. (2011) with some modification and compared it with the control. Mycotoxin levels were detected using thin layer chromatography (Scott *et al*., 1970; Gimeno, 1979; El-kady and Moubasher, 1982).

RESULTS AND DISCUSSION

Yeasts are considered one of the most potent biocontrol agents due to their biology and non toxic properties (Pimenta *et al*., 2009). Several studies have reported that the antagonistic activity of yeasts against fungi may be associated with competition for nutrients and space or adhesion of the cells to the fungal mycelium (Spadaro *et al*., 2002; Spadaro and Gullino, 2004). The results in this study were recorded in Tables (1&2) and showed that *Candida krusei* AUMC 8161 completely inhibited the growth and toxin formation of all the 11 tested toxigenic isolates. This is supported by reports of *Candida* species in

Table (1): The inhibitory effect (%) of some yeasts strains on growth and toxins formation by the standard toxigenic fungal strains grown on potato-dextrose liquid medium, individually, at 28 ºc for 10 days

Toxigenic fungal isolates / Yeast isolates	*A. parasiticus* CBS 571.65 (aflatoxin producer)		*A. ochraceus* CBS 589.68 (ochratoxin producer)		*F. equeseti* CBS 406.86 (zearalenone producer)		*P. griseofulvum* CBS 315.63 (patulin producer)		*P.scabrosum* CBS 530.97 (fumigillin producer)		*Phaeosphaeria nodorum* CBS 438.87 (alternariol producer)	
	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %
control	+5	0	+4	0	+5	0	+5	0	+3	0	+4	0
Candida krusei AUMC 8161	0	100	0	100	0	100	0	100	0	100	0	100
Pichia anomala AUMC 2674	0	100	+1	100	0	100	+5	0	0	100	0	100
Pichia guilliermondii AUMC 2663	+2	80	+1	80	+1	100	+1	60	+1	100	0	100
Saccharomyces cerevisiae AUMC 3875	+2	90	0	100	0	100	+2	80	+2	80	0	100

Table (2): The inhibitory effect (%) of some yeasts strains on growth and toxins formation of some local toxigenic fungal strains grown on potato- dextrose liquid medium, individually, at 28 ºC for 10 days.

Toxigenic fungal strains / Yeast strains	*Alternaria alternata* 5 (alternariol producer)		*A. flavus* 30 (aflatoxin producer)		*A. nidulans* 69 (sterigmatocystin producer)		*A. ochraceus* 76 (ochratoxin producer)		*P. digitatum* 131 (patulin producer)	
	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %
control	+5	0	+5	0	+5	0	+4	0	+5	0
Candida krusei AUMC 8161	0	100	0	100	0	100	0	100	0	100
Pichia anomala AUMC 2674	0	100	+1	100	0	100	+2	90	+1	100
Pichia guilliermondii AUMC 2663	+1	100	+3	80	+2	90	+2	80	+2	60
Saccharomyces cerevisiae AUMC 3875	0	100	0	100	+5	90	+4	40	+1	100

the literature such as the inhibition of *Aspergillus flavus* By *C. krusei* (Hua *et al*., 1999) and inhibition of *Fusarium oxysporum* by *Candida steatolytica* (El-Mehalawy, 2004). Korres *et al*. (2011) reported the inhibition of two pathogenic *Fusarium* isolates by *C. krusei* and *K. apis*.

Pichia anomala AUMC 2674 completely inhibited the growth and toxin production by *A. parasiticus* CBS 571.65, *Penicillium scabrosum* CBS 530.97, *Fusarium equiseti* CBS 406.86, *Phaeosphaeria nodorum* CBS 438.87, *Alternaria alternata* 5 and *A. nidulans* 69 and strongly reduced the growth as well as toxin formation by the other tested toxigenic fungi. Petersson and Schnürer (1995) reported the ability of *P. anomala* to restrict fungal growth and their sporulation on agar plates. Masoud *et al*. (2005) found that *P. anomala* and *Pichia kluyveri* inhibited the production of ochratoxin by *A. ochraceus* on malt extract agar medium and on coffee agar medium.

Pichia guilliermondii AUMC 2663 completely inhibited the growth of *Phaeosphaeria nodorum* CBS 438.87 and strongly reduced the growth of the other 10 toxigenic fungi and highly reduced their toxins formation. Several strains of *P. guilliermondii* have been shown to have biocontrol efficacy against infection by various fungi on citrus fruit, grapefruit, apples, pears, table grapes and strawberries (Droby *et al*., 1997; Arras *et al*., 1999).

Saccharomyces cerevisiae AUMC 3875 completely inhibited the growth of *A. ochraceus* CBS 589.68, *Fusarium equiseti* CBS 406.86, *Phaeosphaeria nodorum* CBS 438.87, *Alternaria alternata* 5 and *A. flavus* 30 and strongly reduced the growth of the other toxigenic fungi. Previous studies demonstrated that *S. cerevisiae* RC008 and RC016 were capable of inhibiting the development of aflatoxigenic *A. parasiticus* strain in different environmental conditions *in vitro* (Armando *et al*., 2011, 2012a, b). *Saccharomyces cerevisiae* have been extensively studied, for their detoxifying potential on aflatoxins, ochratoxin, and zearalenone (Santin *et al*., 2003; Yiannikouris *et al*., 2003). Armando *et al*. (2013) reported that *S. cerevisiae* RC008 and RC016 were able to inhibit *A. carbonarius* and *F. graminearum* growth and reduced ochratoxin and zearalenone. Stinson *et al*. (1978) reported complete degradation of patulin during fermentation of apple juice by *S. cerevisiae*.

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