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CIÊNCIAS BIOLÓGICAS:

Gênese na formação multidisciplinar

Alana Maria Cerqueira de Oiveira
(Organizadora)

Atena Editora
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APRESENTAÇÃO

O Livro “Ciências biológicas: Gênese na formação multidisciplinar 2”, traz ao leitor vinte capítulos de relevada importância na área de Genética, Citogenética, Imunologia, Parasitologia, Química medicinal, Saúde pública e Ecologia. Entretanto, caracteriza-se como uma obra multidisciplinar que engloba diversas áreas da Ciências biológicas.

Os capítulos estão distribuídos em temáticas que abordam de forma categorizada e multidisciplinar a Ciências biológicas , as pesquisas englobam estudos de: mapeamentos genético, citogenético, sequenciamento, genética e educação ,análises forenses , doenças genética, eugenesia clássica, engenharia genética, análise por PCR, cultura de células de linfoma e leucemia, saúde mental, resposta imune, vacinação contra a covid-19, vírus Sars-Cov-2, métodos de extração de lipídios ,levantamento taxonômico, morfologia vegetal, eficiência de inseticidas , química medicinal, cromatografia líquida de alta eficiência (CLAE), espectroscopia de infravermelho (IV) e espectrometria de massas (EM), problemática ambiental e de saúde pública, poluentes emergentes e biodiesel.

A obra foi elaborada primordialmente com foco nos profissionais, pesquisadores e estudantes pertencentes às áreas de Ciências biológicas e Ciências da Saúde e suas interfaces ou áreas afins. Entretanto, é uma leitura interessante para todos aqueles que de alguma forma se interessam pela área.

Cada capítulo foi elaborado com o propósito de transmitir a informação científica de maneira clara e efetiva, em português, inglês ou espanhol. Utilizando uma linguagem acessível, concisa e didática, atraindo a atenção do leitor, independente se seu interesse é acadêmico ou profissional.

O livro Ciências biológicas: Gênese na formação multidisciplinar 2”, traz publicações atuais e a Atena Editora traz uma plataforma que oferece uma estrutura adequada, propícia e confiável para a divulgação científica de diversas áreas de pesquisa.

Alana Maria Cerqueira de Oliveira

SUMÁRIO

CAPÍTULO 7

POTENTIAL OF *Saccharomyces cerevisiae* IN *Fusarium graminearum* ANTIBIOSIS AND ZEARALENONE DETOXIFICATION

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ABSTRACT: Considering the serious economic losses and human and animal health hazards caused by the natural occurrence of zearalenone (ZEA), a toxic secondary metabolite produced by *Fusarium graminearum*, the objective of this study was to evaluate the ability of five *Saccharomyces cerevisiae* strains isolated from baker's yeast to inhibit fungal growth and remove ZEA *in vitro*. The antifungal activity of the *Saccharomyces cerevisiae* strains was evaluated against two strains of *F. graminearum* (FRS 26 and FSP 27). All the yeast strains were able to inhibit *F. graminearum* growth, with inhibition rate ranging from 48.2 ± 1.6 to 73.2 ± 4.0 % (FRS 26) and from 44.2 ± 2, 5 to 63.1 ± 1.5 % (FSP 27). Yeast volatile compounds significantly reduced *F. graminearum* FRS 26 growth ($p < 0.05$). On the other hand, there was no significant difference ($p < 0.05$) in growth reduction of both strains of *F. graminearum* by diffusible compounds compared to the control. Furthermore, the ScE strain reduced the mycelial growth of both strains *of F. graminearum* in corn seeds and caused morphological changes in *F. graminearum* FSP 27, such as thinner hyphae. *Saccharomyces cerevisiae* strains also were able to remove ZEA *in vitro*, and the highest reduction in the ZEA concentration was shown by non-viable cells of the ScC (84.8 ± 0.0%) and ScD (86.3 ± 2.0 %) strains. *S. cerevisiae* strains inhibited *F. graminearum* growth and showed potential for ZEA removal *in vitro*. It can be included in a new product in animal feed, reducing economic losses and minimizing the risk of ZEA carry-over to human food.

KEYWORDS: Adsorption; yeast; antifungal activity; decontamination.

POTENCIAL DE *Saccharomyces cerevisiae* NA ANTIBIOSE DE *Fusarium graminearum* E NA DETOXIFICAÇÃO DE ZEARALENONA

RESUMO: Considerando as grandes perdas econômicas e os riscos à saúde humana e animal causados pela ocorrência natural de zearalenona (ZEA), um metabólito secundário tóxico produzido por *Fusarium graminearum*, o objetivo deste estudo foi avaliar a capacidade de cinco cepas de *Saccharomyces cerevisiae* isoladas de fermento de pão em inibir o crescimento fúngico e remover ZEA *in vitro*. A atividade antifúngica de *Saccharomyces cerevisiae* foi avaliada contra duas cepas de *F. graminearum* (FRS 26 e FSP 27). Todas as cepas de leveduras foram capazes de inibir o crescimento de *F. graminearum*, com taxas de inibição variando de 48,2 ± 1,6 a 73,2 ± 4,0 % (FRS 26) e de 44,2 ± 2,5 a 63,1 ± 1,5 % (FSP 27). Os compostos voláteis das leveduras reduziram significativamente o crescimento de *F. graminearum* FRS 26 ($p < 0,05$). Por outro lado, não houve diferença significativa ($p < 0,05$) na redução do crescimento de ambas as cepas de *F. graminearum* por compostos difusíveis em comparação ao controle. Além disso, a cepa ScE reduziu o crescimento micelial de ambas as cepas de *F. graminearum* em sementes de milho e causou alterações morfológicas de *F. graminearum* FSP 27, como o afinamento das hifas. As cepas de Sa*ccharomyces cerevisiae* foram capazes de remover a ZEA *in vitro*, sendo que a maior redução da concentração de ZEA foi apresentada por células não viáveis das cepas ScC (84,8 ± 0.0%) e ScD (86,3 ± 2,0 %). As cepas de *Saccharomyces cerevisiae* inibiram o crescimento de *F. graminearum* e apresentaram potencial para a remoção de ZEA *in vitro*, podendo ser incluídas em um novo produto na alimentação animal, reduzindo as perdas econômicas e minimizando o risco de transferência de ZEA para a alimentação humana.

PALAVRAS-CHAVE: Adsorção, levedura, atividade antifúngica, descontaminação.

1 I INTRODUCTION

Brazil is the third largest corn producer worldwide, with a production of over 110 million tons in the 2019/20 harvest season. Paraná State is the second largest national corn producer, representing 17% of national production in the 2018/19 harvest season (USDA, 2020).

In Brazil, approximately 45 % of corn production is intended for swine and poultry feed production (ABIMILHO, 2018).

Because of its nutritional quality, corn becomes a good substratum for toxigenic fungi contamination, which produces toxic secondary metabolites (mycotoxins) (FERROCHIO *et al*., 2013). Zearalenone (ZEA), a lactone of β-resorcylic acid, is a mycotoxin produced mainly by *Fusarium graminearum* and commonly detected in wheat and corn. Pigs are the most sensitive animals to the ZEA toxic effects, which are related to their estrogenic properties such as infertility, vulvovaginitis and rectal and vaginal prolapse (KUIPER *et al*., 1998; MARIN *et al*., 2013).

The main control strategy to minimize pre-harvest mycotoxin contamination is fungicide application, but its use can be hazardous to human and animal health and to the environment. The increasing demand for pesticide-free agricultural products requires

cleaner and safer technologies (LIU *et al*., 2011). Biological control of pre- and post-harvest diseases is an effective and safe alternative for minimizing mycotoxins in the food production chain (CHANCHAICHAOVIVAT *et al*., 2007).

Yeasts can produce extracellular compounds with antimicrobial activity called "killer" toxins (CHANCHAICHAOVIVAT *et al*., 2007), but the most probable mechanism involved in biological control of filamentous fungi is competition for nutrients and space (CAVAGLIERI *et al*., 2004). Several studies have shown the potential of yeasts to inhibit fungal growth such as *Fusarium graminearum* (ARMANDO *et al*., 2013) and *F. fujikuroi* (MATIĆ *et al*., 2014), *Geotrichum citri-aurantii* (KLEIN and KUPPER, 2018) and *Penicillium italicum* (CUNHA *et al*., 2018).

A post-harvest approach to minimize mycotoxin contamination is detoxification of contaminated cereals (ZINEDINE *et al*., 2007). The use of adsorbents as additives in animal feed reduces the risk of mycotoxicosis and minimizes mycotoxin carry-over in products of animal origin (meat, eggs, and milk) (SABATER-VILAR *et al*., 2007).

Several authors have reported the use of yeast for ZEA, ochratoxin A and aflatoxin B_1 detoxification (FAUCET-MARQUIS *et al*., 2014; FRUHAUF *et al*., 2012; JOANNIS-CASSAN *et al*., 2011). The cell wall of *Saccharomyces cerevisiae* has an inner layer constituted of β-D-glucans and chitin, and an outer layer formed by glycosylated mannoproteins. The mannoprotein and glucan provide numerous readily accessible binding sites with different connection mechanisms, such as hydrogen bonds, hydrophobic or ionic interactions (YIANNIKOURIS *et al*., 2004; HUWIG *et al*., 2001).

Although yeasts have been applied as biological control agents, there are few studies about the ability of yeasts to inhibit *F. graminearum* growth. Considering the potential of yeasts to inhibit filamentous fungi growth and mycotoxin removal, as well as the demand for pesticide-free products, the objective of this study was to evaluate *S. cerevisiae* application in *Fusarium graminearum* antibiosis and ZEA removal.

2 | MATERIALS AND METHODS

2.1 Yeast and fungal strains

Saccharomyces cerevisiae strains were obtained from fresh baker's yeasts which were acquired in Londrina markets, Paraná, Brazil. A total of five yeast strains were obtained and denominated ScA, ScB, ScC, ScD and ScE.

Two *Fusarium graminearum* strains (FSP 27 and FRS 26) used as fungal test were provided by the Mycological collection of the Toxigenic Fungi and Mycotoxins Laboratory of the Department of Microbiology, Biomedical Sciences Institute, University of São Paulo (São Paulo-Brazil).

2.2 Antifungal activity evaluation

Antifungal activity was evaluated according to Zhao *et al*. (2014), Velluti *et al*. (2004) and Matić *et al*. (2014) with some modifications. Yeast cells were cultured in YPD (yeast, peptone, dextrose) agar, incubated at 25 ± 1 °C for 24 h. Then an aliquot of the inoculum subculture was transferred to Erlenmeyer flasks containing 50 mL YPD broth, which was incubated at 25 ± 1 °C, 150 rpm for 48 h. Yeast cell concentrations were standardized at 10^5, 10^6 and 10^7 cells/mL. One millilitre of cell suspension was homogenized and poured into 20 mL Sabouraud agar. An 8 mm agar plug from an actively grown mycelium of *F. graminearum* FRS 26 and *F. graminearum* FSP 27 was placed on the centre of the Petri dish. All the plates were incubated at 25 ± 1 °C for 7 days and fungal colony diameters were measured. Experiments were performed in triplicate. The inhibition ratios of *F. graminearum* mycelium growth were calculated according to the following formula:

$$\text{Inhibition ratio (\%)} = [(C - T) / C] \times 100\ \% \qquad \text{(Formula 1)}$$

Where:

C = control colony diameter

T = treatment colony diameter

2.3 Evaluation of the antifungal activity of volatile compounds

The antifungal activity of volatile compounds produced by yeasts was evaluated according to the mouth-to-mouth method (Medina-Córdova *et al*., 2016). An 8 mm disc of fungal growth was inoculated (upper plate) in a Petri dish containing 20 mL potato dextrose agar (PDA). In another Petri dish, 20 mL of YPD agar was added and then an aliquot of 100 mL suspension containing 10^8 yeast cells/mL was spread (lower plate). After medium solidification and microorganism inoculation, the plates were paired, sealed with parafilm, and incubated at 25 ± 1 °C for 7 days. Mouth-to-mouth plates without yeast inoculum were used as controls. Experiments were performed in triplicate. The inhibition ratios of *F. graminearum* mycelium growth were calculated according to the formula described in item 2.2.

2.4 Evaluation of diffusible compound effect on *F. graminearum* growth

Diffusible compounds were evaluated as described by Medina-Córdova *et al*. (2016). In Petri dishes containing PDA, an eight mm disk of *F. graminearum* mycelium with seven-day growth in PDA was placed in each plate, and an aliquot of 10 mL yeast cells (1 x 10^8 cells/mL) 3 cm on the opposite side was inoculated and incubated at 25 ± 1 °C, for 7 days. After the incubation time, the colony growth diameter was measured, and the inhibition ratios of *F. graminearum* mycelium growth were calculated according to the formula described in item 2.2.

2.5 *F. graminearum* mycelium preparation for ultrastructural study

For this experiment, the yeast strain which showed the highest antifungal activity on solid medium was selected. The methodology was adapted from Zhao *et al*. (2014) and Miguel *et al*. (2015). The yeast strain was cultured in Erlenmeyer flasks containing 50 mL YPD broth, and shaken at 150 rpm, 25 ± 1 °C for 24 h. The yeast cell concentration was standardized at 10^5 cells/mL. An 8 mm potato dextrose agar (PDA) plug of *F. graminearum* actively grown for seven days at 25 ± 1 °C was added to the culture. The Erlenmeyer flasks were incubated at 150 rpm, 25 ± 1 °C for five days. Then the culture was stopped by centrifugation (9620 *xg* for 15 min at 0 °C). *F. graminearum* mycelium was collected for scanning electron microscopy analysis.

2.6 Scanning electron microscopy (SEM)

Samples of *F. graminearum* mycelium were fixed with 2.5% glutaraldehyde at 25 °C for 4 h, rinsed 4 times with 0.1 mol/L phosphate-saline buffer (PBS) and subsequently treated with 1 % osmium tetroxide at 20 ± 1 °C for 1 h. The samples were subjected to gradual dehydration in ethanol concentrations (70 %, 80 %, 90 % and 100 %) for 15 min each and dried to the critical point (CPD 030 Critical Point BALTEC Dryer, Leica Microsystems, Liechtenstein). After drying, the samples were glued on stubs using carbon tape and coated with gold (Sputter Coater BALTEC SDC 050, Leica Microsystems, Liechtenstein). The mycelia were analysed using an FEI Quanta 200 scanning electron microscope.

2.7 Evaluation of the antifungal activity on corn seeds

2.7.1 F. graminearum inoculum preparation

Three 8 mm discs of *F. graminearum* growth were inoculated at equidistant points in 250 mL Erlenmeyers flasks, containing 10 g ground corn and 10 mL distilled water were added, then the substrate was autoclaved twice at 121 ± 1 °C for 30 min. The culture was incubated at 25 ± 1 °C for 7 days. Subsequently, 3 g of the culture were added to a tube containing 15 mL saline solution (0.85 % NaCl + 0.01 % Tween 80) and 20 glass beads. The tube was vigorously shaken for 2 min.

2.7.2 Yeast inoculum preparation

Yeast cells were reactivated in YPD agar and incubated at 28 ± 1 °C for 48 h. Then a loop of yeast growth was transferred to 250 mL Erlenmeyer flasks containing 50 mL YPD broth and the culture was incubated at 28 ± 1 °C, 150 rpm for 48 h. The culture was stopped by centrifugation at 9620 *x*g at 4 °C, for 15 min. The yeast cells were suspended in YPD containing 1 % carboxymethylcellulose and standardized at 1 x 10^8 cells/mL concentration.

2.7.3 Yeast and F. graminearum inoculation on corn seeds

Antibiosis in corn seeds was evaluated according to Brasil (2009) following the instructions for the Blotter test to evaluate seed health. Corn seeds were disinfested by washing for 3 min in 1 % sodium hypochlorite followed by 3 consecutive washes with sterile distilled water for 10 min.

Corn seeds were then placed in 150 mm diameter Petri dishes, containing three filter paper disks, previously moistened with sterile distilled water (2.5 times dry paper weight). The plates were incubated at 25 ± 1 °C for 24 h, subsequently the seeds were frozen at -20 ± 1 °C for 24 h. The corn seeds were kept at room temperature for 2 h to proceed with fungus and yeast application.

The corn seeds were immersed in a suspension containing 1 x 10^{8} yeast cells/mL for 30 min. They were transferred to 150 mm diameter Petri dishes containing three filter paper disks previously moistened as described above. An aliquot of 30 mL *F. graminearum* mycelia suspension was applied to each seed.

The corn seeds were arranged individually, 1-2 cm apart, in 150 mm Petri dishes, on three layers of moistened filter paper disks, as previously described. The Petri dishes were placed under white fluorescent light bulbs in a BOD with 12-h photoperiod at 28 ± 2 °C, for 5 days.

The results were classified by means of a scale of points adapted from the method described by Sharma (2011): seeds without fungal growth = 0; seeds with growth in the germ = 2; seeds with growth in the pericarp = 3; seeds with total growth = 4. Percent inhibition of fungal growth was calculated by Formula 2:

$$\text{Inhibition Rate (\%)} = [(Po-Pn) / Po] \times 100\% \qquad \text{(Formula 2)}$$

Where:

Po = total fungal growth score in the control

Pn = fungal growth score in the treatment

2.8 Detoxification assay

The ZEA detoxification assay was performed according to Armando *et al*. (2012) and Bordini *et al*. (2014) with some modifications. Yeast strains were grown in YPD agar and incubated at 25 ± 1 °C for 24 h. Subsequently, the culture was transferred to Erlenmeyer flasks containing 50 mL sterile YPD broth and incubated at 25 ± 1 °C, 150 rpm for 24 h. The experiments were divided into two groups: viable cells (no heat treatment) and unviable cells (heated at 121 °C for 15 min). Afterwards the cell suspensions (viable and unviable) were centrifuged (9620 *xg* at 0 °C for 15 min). The resulting pellet was washed three times with PBS solution, pH 7.4 and four times using ultrapure sterile water. The suspension was standardized at 10^{8} cells/mL in a final volume of 25 mL. This suspension was centrifuged; the pellet was suspended in 10 mL PBS (pH 7.4) to a ZEA final concentration of 500 ng/mL. The assays were then incubated at 37 °C, 250 rpm for 1 h. The experiment was stopped

by centrifugation (9620 *xg* at 0 °C for 15 min). The supernatant was used to determine the ZEA adsorbed.

The controls (without yeast) were prepared simultaneously and the ZEA adsorbed was calculated subtracting the concentration determined in the controls and the supernatant of tests with yeast cells, according to the formula:

$$\text{Adsorption ratio (\%)} = [(Z - Z^t) / Z] \times 100\ \% \qquad \text{(Formula 3)}$$

Where:

Z = ZEA concentration detected in the control (ng/mL)

Z^t = ZEA concentration detected in the supernatant of adsorption tests (ng/mL)

2.9 Zearalenone determination

The supernatant was subjected to clean-up with immunoaffinity column (ZearalaTest®; VICAM, Milfort, MA, USA), according to the manufacturer's instructions (with adaptations). An aliquot of 5 mL of the supernatant was diluted 1: 5 with ultrapure water, then 10 mL of this dilution was applied to the immunoaffinity column. The column was washed with ultrapure water, and ZEA was eluted with 1.5 mL methanol, dried under nitrogen stream at 45 °C and stored at - 20 °C.

ZEA was determined according to Bordini *et al*. (2014). The sample residues were dissolved in 200 mL methanol: water (70: 30, v/ v) and 20 mL were injected into a reversed phase high performance liquid chromatography system (Shimadzu LC-10 AD pump and RF-10A XL fluorescence detector, Shimadzu, Tokyo, Japan), using a C-18 Luna Phenomenex column (250 x 4.6 mm, 5 mm, Scharlau, Barcelona, Spain). The excitation and emission wavelengths were 280 and 460 nm, respectively. The mobile phase was methanol: water (70: 30, v/ v) at a flow rate of 1 mL/min.

The detection and quantification limits were 1.3 ng/mL and 2.35 ng/mL, respectively, defined as 3-fold and 5-fold the standard deviation of 7 replications of an unspiked sample (INMETRO, 2007).

2.10 Statistical analysis

Differences in the mean inhibition ratio of yeast strains against *F. graminearum* isolates were evaluated statistically using ANOVA followed by the Tukey multiple comparison test ($p < 0.05$). Differences in mean ZEA removal (%) were evaluated statistically using ANOVA followed by the Tukey multiple comparison test and differences were assessed in mean ZEA removal (%) between viable and unviable yeast cells using the t test ($p < 0.05$).

3 I RESULTS AND DISCUSSION

Several researchers have studied the antagonistic effect of yeast on *Fusarium fujikuroi*, *F. graminearum* and *Fusarium guttiforme* (ARMANDO *et al*., 2013; KORRES *et al*., 2011; MATIĆ *et al*., 2014; SCHISLER *et al*., 2015).

Walker *et al*. (1995) associated the antagonistic activity of yeasts with the ability to synthesize killer toxins. Other authors associated the antagonistic effect with competition for nutrients (ARMANDO et al., 2013). The mechanisms of antimicrobial activity include antibiosis, competition, parasitism, and extracellular production of substances (KÖHL *et al*., 2015; SCHISLER *et al*., 2015).

The antifungal activity of yeast strains against *F. graminearum* FRS 26 and FSP 27 is shown in Tables 1 and 2, respectively. The yeast strains showed different inhibition degrees against *F. graminearum* FRS 26 (from 48.2 ± 1.6 to 73.2 ± 4.0 %) and *F. graminearum* FSP 27 (from 44.2 ± 2.5 to 63.1 ± 1.5 %). Regarding *F. graminearum* FRS 26, all the yeast strains showed the highest inhibition rate at 10^7 cells /mL. For the ScA, ScB and ScD strains, the inhibition rate was proportional to the cell concentration, but for the ScC and ScE strains the inhibition rate did not differ significantly between 10^5 and 10^6 cells/mL. Regarding *F. graminearum* FSP 27, all the strains also showed the highest inhibition rate at 10^7 cells/mL, but there was no significant difference among the cell concentrations for the ScA, ScC and ScE strains. These results were similar to those reported by Korres *et al*. (2011) who demonstrated that *Candida krusei* BT0701 inhibited 75.8 % of *Fusarium guttiforme* E-261 growth, while *Kloeckera apis* BT0703 showed 41.4 % inhibition of *F. guttiforme* E-261 growth. On the other hand, Armando *et al*. (2013) reported that *F. graminearum* growth *was* completely inhibited by *S. cerevisiae* RC016.

Yeast strains	Cell concentration (cells/ mL)		
	10^5	10^6	10^7
	Inhibition ratio (%)		
ScA	52.4 ± 2.4 $^{b\,C}$	58.9 ± 2.5 $^{b\,B}$	71.4 ± 3.0 $^{b\,A}$
ScB	51.5 ± 3.3 $^{c\,b\,C}$	64.0 ±1.3 $^{a\,B}$	69.9 ± 1.3 $^{a\,b\,A}$
ScC	52.1 ± 0.7 $^{b\,B}$	53.9 ± 1.7 $^{c\,B}$	69.3 ± 7.0 $^{a\,b\,A}$
ScD	48.2 ± 1.6 $^{c\,C}$	51.8 ± 1.6 $^{c\,B}$	65.5 ± 1.5 $^{a\,b\,A}$
ScE	56.0 ± 0.9 $^{a\,B}$	57.7 ± 0.9 $^{b\,B}$	73.2 ± 4.0 $^{a\,A}$

* Means followed by different lowercase letter in the same column are significantly different by the Tukey test ($p < 0.05$) for each yeast strain.

* Means followed by different uppercase letter in the same line are significantly different by the Tukey test ($p < 0.05$) among the three cell concentrations of the same yeast strain.

Table 1. Inhibition ratio (%) of yeast strains at different cell concentrations against *F. graminearum* FRS 26.

Yeast strain	Cell concentration (cells/ mL)		
	10^5	10^6	10^7
	Inhibition ratio (%)		
ScA	54.3 ± 0.6 $^{a\ B}$	56.3 ± 3.1 $^{a\ B}$	60.1 ± 1.8$^{a\ A}$
ScB	44.2 ± 2.5 $^{c\ C}$	56.3 ± 4.6 $^{a\ B}$	62.1 ± 2.5 $^{a\ A}$
ScC	49.2 ± 3.1 $^{b\ B}$	53.3 ± 1.1 $^{a\ A}$	56.1 ± 1.5 $^{b\ A}$
ScD	46.7 ± 2.6 $^{b\ c\ C}$	53.5 ± 2.3 $^{a\ A\ B}$	61.1 ± 3.1$^{a\ A}$
ScE	56.8 ± 1.2 $^{a\ B}$	58.1 ± 3.4 $^{a\ B}$	63.1 ± 1.5$^{a\ A}$

* Means followed by different lowercase letter in the same column are significantly different by the Tukey test ($p < 0.05$) for each yeast strain.

* Means followed by different uppercase letter in the same line are significantly different by the Tukey test ($p < 0.05$) among the three cell concentrations of the same yeast strain.

Table 2. Inhibition ratio (%) of yeast strains at different cell concentrations against *F. graminearum* FSP 27.

The antifungal activity of volatile compounds produced by yeast strains against *F. graminearum* FRS 26 and FSP 27 is shown in Table 3. Volatile compounds of all the yeasts reduced *F. graminearum* growth from 30.4 % ± 6.4 to 34.6 % ± 5.5 (FRS 26) and from 3.4 % ± 4.8 to 26.5 % ± 6.3 (FSP 27), but only FRS 26 growth reduced significantly in relation to the control ($p < 0.05$). The inhibition of fungal growth by volatile compounds was higher than that reported by Ferraz *et al*. (2016), but lower than that reported by Medina-Córdova *et al*. (2016) and Grzegorczyk *et al*. (2017). Ferraz *et al*. (2016) evaluated 95 yeasts and only one inhibited less than 10.0 % *Geotrichum citri-aurantii* growth by volatile compound production. Medina-Córdova *et al*. (2016) demonstrated that the volatile compounds produced by *Debaryomyces hansenii* inhibited *Fusarium proliferatum* and *Fusarium subglutinans* growth by 54.2 and 43.5 %, respectively, in comparison with the control ($p < 0.05$). Grzegorczyk *et al*. (2017) reported that *Wickerhamomyces anomalus* inhibited 47.3 % and 98.0 % of *Monilinia fructigena* and *Monilinia fructicola* growth by volatile compound production.

Yeast strain	*F. graminearum* FRS 26		*F. graminearum* FSP 27	
	Mycelial growth (mm)	Inhibition ratio (%)	Mycelial growth (mm)	Inhibition ratio (%)
ScA	56 ± 3 ^{B}	32.4 ± 3.7 ^{a}	53 ± 5 ^{A}	26.5 ± 6.3 ^{a}
ScB	55 ± 4 ^{B}	34.6 ± 5.5 ^{a}	65 ± 6 ^{A}	10.1 ± 9.3 ^{a}
ScC	57 ± 3 ^{B}	31.6 ± 4.6 ^{a}	57 ± 6 ^{A}	22.1 ± 8.8 ^{a}
ScD	57 ± 5 ^{B}	31.8 ± 3.5 ^{a}	70 ± 4 ^{A}	3.4 ± 4.8 ^{a}
ScE	58 ± 6 ^{B}	30.4 ± 6.4 ^{a}	58 ±8 ^{A}	19.8 ± 13.0 ^{a}

Control[1]	81 ± 6 [A]	-	73 ± 2 [A]	-

* Means followed by different uppercase letter in the same column are significantly different by t-test ($p < 0.05$)

* Means followed by different lowercase letter in the same column are significantly different by the Tukey test ($p < 0.05$) for each yeast strain.

[1] plates without yeast inoculum

Table 3. Inhibition ratio (%) of volatile compounds produced by yeasts on fungal growth.

The antifungal activity of diffusible compounds from yeast strains against *F. graminearum* FRS 26 and FSP 27 is shown in Table 4. Inhibition of FSP 27 ranged from 5.0 ± 8.8 % to 12.8 ± 9.3 %, but there was no statistical difference from the control ($p > 0.05$). Fungal growth inhibition was lower than that reported by Medina-Córdova *et al.* (2016) who stated that diffusible compounds of *Debaryomyces hansenii* inhibited *Aspergillus* sp., *F. proliferatum* and *F. subglutinans* growth in 56.3 %, 38.9 % and 25.9 %, respectively.

There were no significant differences regarding the effect of volatile compounds and diffusible compounds from the yeast strains on fungal growth. *S. cerevisiae* ScE was selected for the study of structural modifications and antibiosis on corn seeds because it showed the highest antibiosis by the agar diffusion method.

Yeast strain	*F. graminearum* FRS26		*F. graminearum* FSP 27	
	Mycelial growth (mm)	Inhibition ratio (%)	Mycelial growth (mm)	Inhibition ratio (%)
ScA	63 ± 6 [A]	-	61 ± 7 [A]	10.6 ± 8.3 [a]
ScB	67 ± 4 [A]	-	60 ± 3 [A]	12.8 ± 9.3 [a]
ScC	62 ± 8 [A]	-	65 ± 6 [A]	5.0 ± 8.8 [a]
ScD	63 ± 11 [A]	-	65 ± 7 [A]	5.5 ± 20.0 [a]
ScE	63 ± 6 [A]	-	61 ± 7 [A]	10.1 ± 13.0 [a]
Control[1]	62 ± 11 [A]	-	69 ± 11 [A]	-

* Means followed by different uppercase letter in the same column are significantly different by the t-test ($p < 0.05$)

* Means followed by different lowercase letter in the same column are significantly different by the Tukey test ($p < 0.05$) for each yeast strain

[1] plates without yeast inoculum

- no inhibition

Table 4. Inhibition ratio (%) of diffusible compounds produced by yeasts on fungal growth.

Evaluation of morphological changes can be applied to the study of the antagonistic mechanism. Some yeasts with antagonistic potential can synthesize enzymes such as

glucanases, chitinases and proteases which cause morphological changes in hyphae (JANISIEWICZ and KORSTEN, 2002). The scanning electron microscopy (SEM) analysis showed that *Saccharomyces cerevisiae* ScE caused defects on hyphae morphology of *F. graminearum* FSP 27 (Figure 1 C) such as thinner hyphae (Figure 1 A). The mycelial organization revealed by SEM also showed an extracellular material around the hyphae. However, it was not possible to observe morphological changes in *Fusarium graminearum* FRS 26 mycelia in the presence of the ScE strain. The results of the mycelial ultrastructural study in addition to the absence of fungal growth inhibition by diffusible compounds suggest that the main antagonistic mechanism involved in *F. graminearum* antibiosis by *S. cerevisiae* ScE is competition for nutrients. On the other hand, Korres *et al.* (2011) reported that *Kloeckera apis* BT0703 and *Candida krusei* BT0701 caused changes in *Fusarium guttiforme* hyphae and were able to adhere to the surface of the hyphae indicating that the mode of action was associated with hyperparasitism.

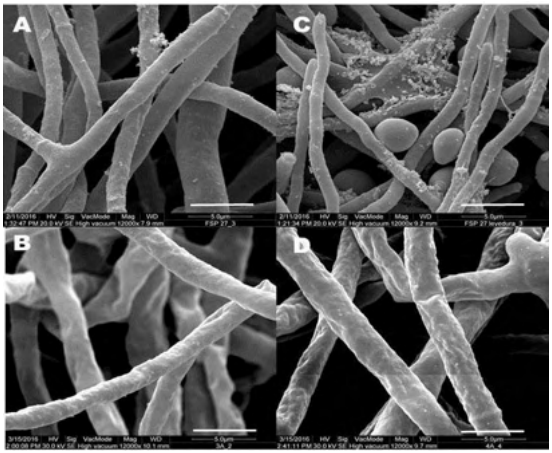

Figure 1. Electron micrographs of *F. graminearum* mycelia FSP 27 and FRS 26 on 5 th day incubation at 25 °C on the absence (control) - A (*F. graminearum* FSP 27) and B (*F. graminearum* FRS 26), and presence (treatment) - C (*F. graminearum* FSP 27) and D (*F. graminearum* FRS 26) of ScE yeast strain. Bar: 5.0 μm.

Corn is susceptible to several soil-borne diseases. *F. graminearum* is an important phytopathogen and when present in the soil can cause root rot (DAGUERRE *et al.*, 2014; PAL *et al.*, 2001). Prolonged use of agrochemicals may harm the ecological environment of soil; therefore, it is necessary to develop biological control methods that minimize the incidence of seed diseases (HE *et al.*, 2019; MATIĆ *et al.*, 2014). The *S. cerevisiae* ScE strain reduced the growth of both strains of *F. graminearum* on corn seeds (Figure 2) compared to the control ($p < 0.05$). The percent growth inhibition was 23.0 ± 4.9 % for FRS 26 and 17.9 ± 2.7 % for FSP 27 (Figure 2). These results are in line with Matić *et al.* (2014). *Pichia guilliermondii* R9, *Metschnikowia pulcherrima* R23 and R26 reduced the infection

rate of *F. fujikuroi* by 14.3 %, 16.7 % and 18.0 %, respectively (MATIĆ *et al*., 2014). These results indicate that *S. cerevisiae*, used as baker's yeast, has potential to prevent soil-borne diseases.

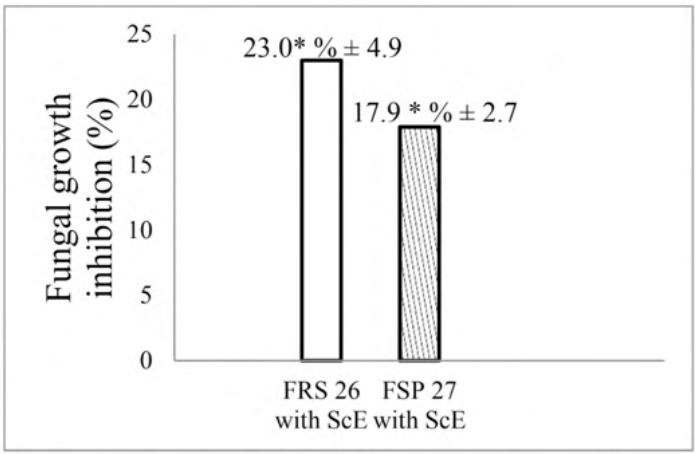

Figure 2. Evaluation of the ScE antagonistic capacity in BALU 761 corn seeds. Data from trials were collected after five days of incubation at 28 °C. Each bar represents the average ± SD of four replicates. The results were classified by means of a scale of points ranging from 0 (seeds without fungal growth) to 4 (seeds with total growth). The percentage of growth inhibition was then calculated by the quotient of the control score difference and the treatment score, divided by the control score multiplied by 100. Mean values significantly different from the control according to the t-test ($p < 0.05$) are indicated by an asterisk.

Some researchers have reported the application of yeast or yeast's cell wall for mycotoxin adsorption (BORDINI *et al*., 2014; FREIMUND *et al*., 2003; FRUHAUF *et al*., 2012). The cell wall of *Saccharomyces cerevisiae* is composed of two layers, comprising mainly mannoproteins, glucan (β-1,3 and β-1,6 glucan polysaccharide) and chitin (polymer of β-1,4 N-acetylglucosamine) linked by covalent bonds (KOGAN and KOCHER, 2007; SHETTY *et al*., 2007). The different ability of the yeast cell wall for mycotoxin adsorption is assigned to glucans present in its structure (HUWIG *et al*., 2001). The interaction between ZEA and β-D-glucans occurs by stereochemical complementarity, involving van der Waals interactions. The hydroxyl and ketone groups of ZEA bind via hydrogen bonds, and other stable molecular forces, to the hydroxyl groups of the single helix structure of (1,3) β-D-glucan (YIANNIKOURIS *et al*., 2004; YIANNIKOURIS *et al*., 2003).

In this study, *in vitro* ZEA removal by viable yeast cells ranged from 67.1 ± 4.1 % (ScE) to 76.0 ± 0.0 % (ScA). There was no significant difference in mean ZEA removal among the viable yeast strains. Unviable cells (heat inactivated) showed ZEA removal ranging from 78.0 ± 0.6 (ScE) to 86.3 ± 2.0 % (ScD) (Table 5). Comparing the ZEA removal (%) by the same *S. cerevisiae* strain, unviable cells of the ScC and ScD strains showed higher ability ($p < 0.05$) than the viable cells. Other strains showed no significant difference ($p > 0.05$) in

mean ZEA removal (%) between viable and unviable cells. This result indicates that ZEA removal from the medium does not occur by enzymatic degradation but by adsorption to the cell wall, since unviable cells were more efficient in ZEA removal. The heat treatment may have altered the conformation of the yeast cell wall, making the hydroxyl groups present in β-D-glucan more available for toxin-glucan complex formation (YIANNIKOURIS *et al*., 2004).

Yeast strains	% ZEA removal	
	Viable cells	Unviable cells[1]
ScA	76.0 ± 0.0 $^{a\,A}$	82.0 ± 2.6 $^{a\,b\,A}$
ScB	75.71 ± 0.8 $^{a\,A}$	80.2 ± 2.3 $^{a\,b\,A}$
ScC	75.6 ± 1.7 $^{a\,B}$	84.8 ± 0.0 $^{a\,b\,A}$
ScD	71.1 ± 4.7 $^{a\,B}$	86.3 ± 2.0 $^{a\,A}$
ScE	67.1 ± 4.1 $^{a\,A}$	78.0 ± 0.6 $^{b\,A}$

* Means followed by a different lowercase letter in the same column are significantly different by the Tukey test ($p < 0.05$) for each yeast strain.

* Means followed by uppercase different letter in the same line are significantly different by the t test ($p < 0.05$)

[1] Unviable cells: heat treatment (121 °C for 15 min)

Table 5. *In vitro* ZEA removal (%) by viable and unviable yeast cells (10^8 cells/mL) at 500 ng/ mL ZEA concentration.

ZEA removal (%) was similar to that reported by Faucet-Marquis *et al*. (2014), but higher than that obtained by Armando *et al*. (2012) and Bordini *et al*. (2014) (pure yeast cell wall). Faucet-Marquis *et al*. (2014) reported maximum values of 75 % ZEA adsorption using 5 mg yeast cell wall derivative (ZEA concentration of 20 mg/mL, at pH 5). Armando *et al*. (2012) evaluated the potential of *S. cerevisiae* strains for ZEA detoxification and reported binding percentage ranging from 56.7 to 74.2 % at 0.5 mg/mL ZEA concentration. Bordini *et al*. (2014) evaluated ZEA adsorption capacity by a mixture of organic (yeast cell wall) and inorganic (activated charcoal) adsorbents. The adsorbent mixture at 75: 25 ratio showed higher efficiency for ZEA adsorption (> 96.1 %) than the pure yeast cell wall (55.7 - 78.1 %).

In summary, the present study showed inhibition of *Fusarium graminearum* growth by *S. cerevisiae* strains and competition for nutrients was the probable mechanism. All the *Saccharomyces cerevisiae* strains showed potential for *in vitro* ZEA removal. *Saccharomyces cerevisiae* is Generally Recognized as Safe (GRAS), therefore there is no risk for its application in food and feed. It can be included in a new product to inhibit *F. graminearum* growth or for ZEA removal in animal feed, reducing economic losses and minimizing the risk of ZEA carry-over to human food.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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