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THE EFFICACY OF *SACCHAROMYCES CEREVISIAE* AND *CANDIDA KRUSEI* IN REDUCTION OF AFLATOXIN B1 THAT PRODUCED BY *ASPERGILLUS FLAVUS IN VITRO*

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ABSTRACT

Aflatoxin B1 (AFB1) is a potent hepatotoxic and hepatocarcinogenic mycotoxin produced by some filamentous fungi like *Aspergillus flavus* and *Aspergillus parasiticus*. This toxin widely contaminates food and feedstuffs causing health problems to the human and animals when consuming the contaminated food. This study compared between two of well-known biocontrol yeasts *Saccharomyces cerevisiae* (S.c.) and *Candida Krusei* (C.k.) *in* vitro to evaluate their efficiency in control *A.flavus* growth and AFB1 reduction. The results revealed a significant ability of both yeasts to inhibit fungal growth area (3.9, 8.14 mm^2) respectively comparing with the control (71.34 mm^2). Both yeast species (Sc. and C.k.) were significantly reduced 250 ng of AFB1 to the non-detectable levels after 48h of incubation in broth culture.

KEYWORDS: Aflatoxin B1 *Aspergillus flavus* and *Aspergillus parasiticus*.

INTRODUCTION

Aflatoxin B1 (AFB1) is an important secondary metabolite produced by several fungal species like *A.flavus*, *A.parasiticus* and some species of *Penicillum spp* (Deepak et al., 2015; Mohammadi et al., 2017). This toxin is well known as a potent carcinogenic compound that causes liver cancer in human and animals (Asim et al., 2011). AFB1 significantly contaminates a wide spectrum of raw and processed food and feedstuffs which make it very hard to control, which leads to a huge loss in agricultural products and their relative industry (Battilani et al., 2016; Sprynskyy et al., 2018). Since the discovery of AFB1 at 1960s, intensive studies were performed to develop physical, chemical and biological methods to

decontaminates AFB1 or at least reduces the contamination to un-hazardous levels (Burgos-Hernández et al., 2002; Mishra & Das, 2010). Biological decontamination of AFB1 tends to be highly promised methods according to their potential reduction, safety and economically low requirements (Burgos-Hernández et al., 2002; Yan-ni YIN et al., 2004). The mechanism of biological control of AFB1 can be determined either by absorption, which can be performed by cell wall components of live or dead microorganism, or by enzymatic degradation by intracellular and/or extracellular enzymes (Azizollahi Aliabadi et al., 2013). Yeasts were considered as a significant biocontrol agents to neutralize AFB1 according to their high absorption ability to AFB1 (Azizollahi Aliabadi et al., 2013; Mohammadi et al., 2017). *S.cervisiae* was reported as highly effective AFB1 binder as their cell wall includes an adhesive net composed of β-1, 3 glucan as a backbone with side chains of same compound covered with highly glycosylated mannoproteins layer (Gonçalves et al., 2014). Furthermore, *S.cervisiae* classified as QPS (Qualified Presumption of Safety) according to EFSA (The European Food Safety Authority), in addition to other beneficial features to the human health (Moslehi-Jenabian et al., 2010). However, *C.krusei* also reported as a safe biological agent significantly suppress *A.flavus* growth and eliminate AFG1 and G2 levels by binding these toxins to the cell wall polysaccharides network (Mohammadi et al., 2017), with some beneficial effects to the human health (Moslehi-Jenabian et al., 2010). The objective of this study was to evaluate and compare the efficiency of the *S.cervisiae* and *C.krusei* individually to reduce AFB1 levels *in vitro*.

MATERIALS AND METHODS

Fungal Isolates

A.flavus AFL14 isolate was isolated and identified morphologically and molecularly from past study (Al-Saad et al., 2016). The yeast *S.crvisiae* was isolated from commercial bakery yeast (Saf-instant, Ozmaya San. A. S., a Lesaffre company, Turkey). The isolate of *C.krusei* was obtained as a gift from Proff. Abdulla Al-Sadoon, University of Basrah, College of Science, Dept. of Biology.

A. flavus growth inhibition

The effect of *S.cervisiae* and *C.krusei* on *A.flavus* AFL14 growth was examined by preparing potato dextrose agar (PDA) medium (PDA, Oxoid, U.K.) and pouring it in 9 cm Petri plates, when the medium cooled down to 40ºC, 1 ml of 10^6 CFU/ml of *S.cervisiae* and *C.krusei* were mixed separately with the medium and left to solidify then incubated at 35ºC for 24h, After

that 0.5 cm discs of one day old *A.flavus* culture where inoculated centrally in the yeasts pre-inoculated plates. A control treatment involved inoculation of 0.5 cm discs in the one-day old *A.flavus* culture centrally of non-yeasts treated PDA plates. The treatments performed in triplicate. The inoculated plates were incubated at 35ºC until the control treatment filled the plates. The growth area of each treatment was estimated using image processing software (UTHSCSA Image Tool, V. 2. 0.) that was developed by University of Texas Health Science Center, San Antonio. The method included scaling the software according to the known object in the Petri plate image, which was the diameter of the plate itself, then pointing the growth zone by selection tool of the program and the software will calculate the selected zone area automatically. Five shots of different sides for each plate was captured and the average of calculated area was considered to ensure accuracy (Barguil et al., 2005; de A Campos et al., 2008; Mamede et al., 2006; Al-saad et al., 2014).

Aflatoxin B1 Reduction

A test tubes contained 8 ml of nutrient broth (NB) medium (NB, Oxoid, U.K.) were autoclaved for 20 min at 121ºC then mixed with 1ml of DMSO (Dimethyl sulfoxide) contains 250 ng/ml of pure AFB1 (Himedia, India). The tubes were inoculated with 1 ml of 10^6 CFU/ml *S. Cervisiae* and *C.krusei* separately and incubated at 35ºC in shaking incubator (150 rpm) for 48h.

AFB1 extraction

AFB1 was extracted according to Al-Saad et al. (2016) with little modification, a 20 ml of chloroform then filtered with Whatman filter paper No. 4. The extract was concentrated with a rotary evaporator to near the dryness, then collected with 5 ml vials and re-filtered with 0.2 Millipore nylon filter 13mm (SMI-Lab Hut LTD) then air-dried overnight. The dried extract was re-dissolved in 1 ml of chloroform to estimate AFB1 reduction.

The TLC plate 10 × 20 cm was activated in oven for 1 h at 120ºC. A 10 µl of each treatment sample and AFB1standard dilution (1ng/µl) were spotted on the TLC plate in a straight line 1.5 cm from the TLC plate base with an interval of 2 cm between each 2 spots. The TLC plate was placed in the running system contained Chloroform: Methanol, (98:2) and when running reached 2 cm from the upper edge, the plate was dried and examined under UV light (360 nm) and photographed by a digital camera (Nikon, Cool PIX P520) for quantitative estimation.

AFB1 reduction was estimated by high-performance thin layer chromatography (HPTLC) that described by Hoeltz et al. (2010) and Hoeltz et al. (2012) that includes running the sample and standard spots via TLC plate, then take 5 shots to the same plate from different positions to obtain accuracy, then analyzing them using Image J software https://imagej.nih.gov/ij/ to estimate AFB1 quantitatively depending on spots illumination measurement.

Statistics

All experiments were carried out in completely randomized design (CRD) and the ANOVA statistical analysis was performed using SPSS ver. 16.0 software (SPSS Inc., 1989-2007).

RESULTS AND DISCUSSION

A.flavus growth inhibition

The growth area results (Fig.1) presented that *A.flavus* AFL14 growth was inhibited significantly by *C.krusei* and *S.crvisiae* treatments (3.9 and 8.14 mm^2) respectively with no significant differences between them comparing with the control treatment (71.34 mm^2). The mechanism of growth inhibition of *A.flavus* could be interpreted either by out-competition of yeast over *A.flavus* as the both yeasts in this study got their chance to colonize the medium and behave as preventive agents, or by production of toxic materials, which are well known as killer toxins (Marquina et al., 2002). The killer toxins were reported to be lethal to other species of yeasts and employed mostly in competition among yeasts together, this may lead to expects that these compounds may be plays an effective role in *A.flavus* radial growth suppression (Marquina et al., 2002; Persons et al., 2013).

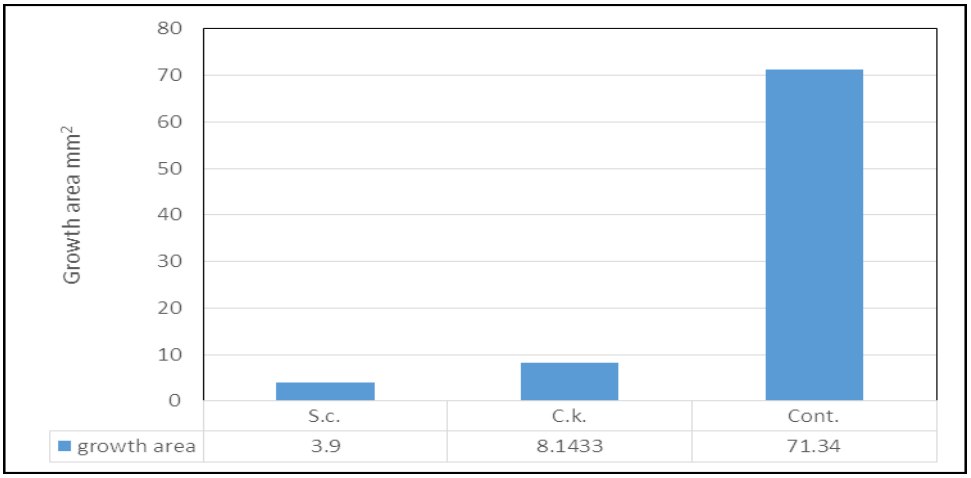

Fig 1: The growth inhibition of *A.flavus* AFL14 by *S.cerevisiae* (S.c.) and *C.krusei* (C.k.). LSD= 9.80.

Aflatoxin B1 Reduction

A significant reduction in the AFB1 levels were recorded after treatment with *C.krusei* and *S.crvisiae* in the broth cultures, both yeasts were significantly (P=0.000) eliminated the AFB1 levels from 250 ng to the non-detected amounts after 48h of incubation (Fig.2) with no significant differences between them (P=1). There are two main mechanisms could be explain the ability of yeasts to decontaminate AFB1, the most approved and well-accepted mechanism is the cell wall binding of AFB, as the yeasts cell wall mostly covered by adhesive layer constructed of polysaccharides and adhesive proteins, significantly able to bind aflatoxins, while the second mechanism represented by enzymatic degradation through production of intracellular and/or extracellular enzymes, which are specifically degrades aflatoxins (Moslehi-Jenabian et al., 2010; Azizollahi Aliabadi et al., 2013; Deepak et al., 2015).

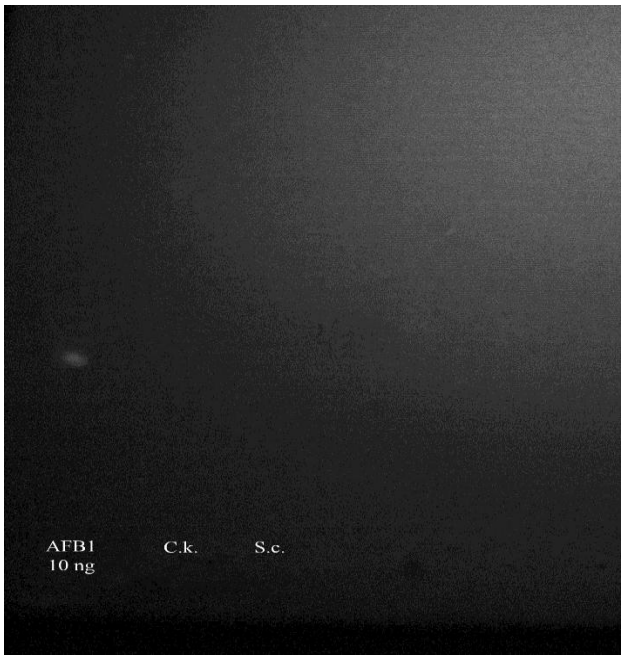

Fig 2: TLC plate showing the activity of *C.krusei* and *S.cerevisiae* in reduction of 250 ng of AFB1 after 48h of incubation: from Left AFB1 standard 10 ng; *C.krusei* +250 ng of AFB1 (C.k.); *S.cerevisiae* + 250 ng of AFB1 (S.C.).

CONCLUSION

S.cervisiae and *C.krusei* are very effective biological control agents can be significantly inhibit *A.flavus* growth and reduce AFB1 levels to the non-detected level. The both yeast species were efficient in decontaminate AFB1*in vitro* and can be considered as biological agents in prevention and/or treatment of contaminated food and feedstuff.

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