POTENCY OF YEAST AS A BIOCONTROL AGENT OF OCHRATOXIN A-PRODUCING FUNGI AND ITS EFFECT ON ARABICA COFFEE TASTE

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ABSTRACT

Biocontrol agents can be used to control mycotoxigenic fungi, which include different species of yeast. The objectives of this research were to select yeast isolates that can inhibit the growth of ochratoxin A (OTA)-producing fungi (*Aspergillus ochraceus* BIO 37310) and to increase the taste of Arabica coffee processed using wet and semi-wet methods. Twenty-two yeast isolates were screened for their antagonistic property against *A. ochraceus* BIO 37310 *in-vitro* using well (dip) test method. The results showed that *Issatchenkia orientalis* = *Candida krusei* (BIO 211287, BIO 211288, and BIO 211289) inhibited *A. ochraceus* BIO 37310. *In-vivo* the highest yeast population was found in coffee beans processed using a semi-wet method inoculated with *I. orientalis* BIO 211288 (46,222 ± 9,576 cfu/g), which was not significantly different from that of the coffee beans inoculated with *I. orientalis* BIO 211287 (36,333 ± 14,000 cfu/g). The three yeast isolates were also able to grow either in coffee beans processed using wet or semi-wet methods inoculated with *A. ochraceus* BIO 37310 and each yeast isolate. Interaction between the three yeast isolates and *A. ochraceus* BIO 37310 resulted in E-type interaction, i.e., the fungus was not able to grow anymore, while the yeasts grew further. The total cupping scores of coffee beans inoculated with the three yeast isolates were higher than those of coffee beans uninoculated and inoculated with commercial lactic acid bacteria. The three yeast isolates could be used as biocontrol agents of *A. ochraceus* BIO 37310 and increase the sensorial quality of coffee beverages.

Keywords: Arabica coffee, biocontrol agent, cupping test, fungi, *Issatchenkia orientalis*, ochratoxigenic, yeast

INTRODUCTION

Indonesia is the fourth largest coffee beans producer in the world after Brazil, Vietnam, and Colombia (Walton 2018) and the second in Southeast Asia after Vietnam. Two kinds of coffee beans are cultivated in Indonesia, i.e., Robusta coffee (*Coffea canephora*) and Arabica coffee (*C. arabica*). Robusta and Arabica coffee contribute about 83% and 17%, respectively, of the total coffee production in Indonesia (GEKI 2018).

During storage, coffee beans can be infested with insects, microorganisms, mites, and rats. Among microorganisms, fungi are the most important cause of the deterioration of stored grains. Fungal infection in grains can cause discoloration, decreases in physical quality and nutritional contents, as well as mycotoxin contamination (Dharmaputra *et al.* 2019).

Ochratoxin A (OTA) contamination in coffee beans has recently become very important as some consumer countries have imposed their maximum tolerable limits (MTL) of the presence of OTA. OTA is a potent nephrotoxic mycotoxin that has been linked to kidney problems in both livestock and human population (Clark & Snedeker 2006). BPOM (2018) has determined the MTL of OTA in coffee powder and *kopi sangrai* (roasted coffee) at 5 ppb, while the MTL of OTA in instant coffee is determined to be at 10 ppb. In Brazil the maximum limit allowed for OTA in roasted coffee is 5 ppb and ground coffee is 10 ppb (European Commission 2010).

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In tropical regions OTA is mainly produced by *Aspergillus carbonarius*, *A. niger*, and *A. ochraceus*, while in sub-tropical regions it is produced by *Penicillium verrucosum* (Bui-Klimke & Wu 2015). *Penicillium citrinum* is the dominant fungus found in Arabica coffee beans collected from farmers, collectors, and traders, while *A. niger* is the dominant fungus found in coffee beans collected from exporters in Tana Toraja Regency, North Toraja Regency, and Makassar Municipality, South Sulawesi Province, Indonesia (Dharmaputra *et al.* 2019).

Biocontrol using antagonistic microorganisms has been an efficient alternative method for controlling OTA producing fungi, thus reducing the use of chemical compounds (Janisiewics & Korsten 2002). According to Korsten (2006) there is a variety of microorganisms which may be used as biocontrol agents against mycotoxigenic fungi, including different species of yeasts, fungi, and bacteria. Due to the positive findings regarding the use of these microbial antagonists, biocontrol agents have been gaining popularity worldwide.

Yeasts' inherent characteristics, such as fast growth, fruit surface colonization, and ability to deprive nutrients from pathogens (through competition), have placed these organisms as one of the most suitable biocontrol agents (Richard & Prusky 2002). Masoud *et al.* (2004) and Vilela *et al.* (2010) also reported that *Pichia kluyveri, P. anomala, Hanseniaspora uvarum, Saccharomyces cerevisiae, Debaryomyces hansenii,* and *Torulaspora delbrueckii* were the most yeast species found during coffee processing.

In other research, mycelial growth of *A. ochraceus* was inhibited by yeast with a high inhibitory effect of 53%. *Pichia anomala* CCMA0148 and *Saccharomyces cerevisiae* CCMA0159 have the highest inhibition rate of all fungal strains growth. It means that these yeasts were potential biocontrol agents in the tested condition (de Souza *et al.* 2017). Pereira *et al.* (2014) reported that as many as 144 yeasts were collected naturally from fermented coffee beans, i.e., *Pichia fermentans, P. kluyveri, P. guilliermondii, P. caribbica, Candida glabrata, C. quercitrusa, Saccharomyces* sp., and *Hanseniaspora opuntiae.*

In Indonesia, coffee fruits are fermented after harvesting. There are three methods of coffee bean processing, i.e., dry, wet, and semi-wet processing methods. According to Kementerian Pertanian Republik Indonesia (2012), **dry** processing method is carried out by harvesting ripe wet cherry beans. The wet beans are then sun-dried for fourteen days until the moisture content reaches ± 10% (dried cherries), at which point the husk and hull of the coffee beans are then shelled. **Wet** processing method is conducted by shelling ripe wet cherry beans. This results in wet green beans with hull, which are then fermented for one night and rinsed to eliminate mucus. Subsequently, the wet green beans are sun-dried for seven days until the moisture content reaches ± 10% (dried green beans), at which point the hull of the beans are then shelled (green beans). **Semi-wet** processing method is carried out by shelling ripe wet cherry beans. Then, the wet green beans are fermented for one night and washed. The wet green beans are then sun-dried for one day until the moisture content reaches ± 40%, at which point the hull of the coffee beans are then shelled. After shelling, the beans are further sun-dried for five days until the moisture content reaches ± 10%.

Using a biocontrol agent, such as yeast, is useful to inhibit toxigenic fungal growth which can cause adverse effect on human health. This research is one of new and innovative studies which has some benefits, especially for food safety. Biocontrol agents can be obtained from the antagonist yeast which cannot cause allergic cells or toxins. This study aims to select yeast isolates that can inhibit the growth of OTA-producing fungi and improve the taste of wet and semi-wet processed Arabica coffee.

MATERIALS AND METHODS

OTA-Producing Fungus, Yeast Isolates, and Arabica Coffee Beans

One isolate of *A. ochraceus* BIO 37310 was used as OTA-producing fungus. Twenty-two yeast isolates (KA, KA2, KB, KB2, KC, KD, *Endomyces decipiens* BIO 131215, *E. fibuliger* BIO 132216, BIO 132217, BIO 132218, BIO 132219, BIO 132220, *Issatchenkia orientalis* (= *C. krusei*) BIO 211285, BIO 211286, BIO 211287, BIO 211288, BIO 211289, BIO 211290, BIO 211291,

Saccharomyces cerevisiae BIO 341363, BIO 341364, and BIO 341365) were screened for their antagonistic property against *A. ochraceus* BIO 37310 *in-vitro*. All isolates were cultured on Potato Dextrose Agar (PDA) media. The fungus and the yeasts were obtained from the Culture Collection Phytopathology Laboratory, SEAMEO BIOTROP, Bogor, Indonesia. Arabica coffee beans Grade 1 were obtained from CV Frinsa Agrolestari, Pangalengan, Bandung.

Screening for Antagonistic Yeasts against *A. ochraceus* BIO 37310

Screening for antagonistic yeasts against *A. ochraceus* BIO 37310 was conducted using a well (dip) test method (Dan *et al.* 2003). Five pieces (5 mm in diameter) of pure culture of each yeast isolate were cultured on 25 mL of Nutrient Yeast Dextrose Broth (NYDB) in an Erlenmeyer flask (volume 100 mL), incubated at 28 ± 2 °C for seven days, and shaken using a KOTTERMAN 4020 shaker for one hour every 24 hours with speed of 150 rpm.

The yeast cells were precipitated by a centrifugation technique using a microcentrifuge 200 R (a kind of centrifuge rotor having a fixed-angle rotor) at 7,000 rpm for 15 minutes and rinsed twice using sterile distilled water. They were then resuspended in sterile distilled water until the concentration reached 5 x 10^8 cell/mL. The yeast cell was calculated using a haemacytometer.

A well (6 mm in diameter) was prepared using a cork borer at the center of a Potato Dextrose Agar (PDA) containing 15% Arabica coffee bean extract on a Petri dish (9 cm in diameter). As much as 20 µL of 5x10^8 cell/mL of yeast cell suspension was placed in the well. Those Petri dishes were left for two hours to allow the cell suspension to diffuse into the well. Furthermore, each 20 µL of 5 × 10^6 conidia/mL of the OTA-producing fungus was placed in the well.

As a control, the well was not inoculated with yeast cell suspension. Three replicates (= three Petri dishes) were used for each treatment (each yeast isolate), including the control. The dishes were incubated at room temperature (28 ± 2 °C) under dark conditions. The growth of *A. ochraceus* BIO 37310 in each Petri dish was observed based on the presence of their colonies after seven days of incubation. The yeast isolate which inhibited the total growth of *A. ochraceus* BIO 37310 was used in the biocontrol assay of *A. ochraceus* BIO 37310 *in-vivo*.

Biocontrol Agent of OTA-Producing Fungi using Yeasts *In-Vivo*

Five hundred grams of Arabica coffee beans with hull (derived from coffee after harvest, shelled, and washed) were placed on a tray (30 x 40 x 5 cm). The beans were inoculated with 10 mL suspension of each yeast isolate (5 x 10^6 cell/mL) (Evangelista *et al.* 2014) and left for 12 hours. Afterwards, the coffee beans were inoculated with 10 mL (5 x 10^4 conidia/mL) of *A. ochraceus* BIO 37310.

As a control, coffee beans with hull were not inoculated with the yeasts. Coffee beans with hulls were also inoculated with commercial lactic acid bacteria. After inoculation, coffee beans were placed on porous trays. Three replicates (= three trays) were used for each treatment (including the control).

In the wet processing method, coffee beans were sun-dried for five days until the moisture content reached 9 to 10%, at which point the hulls were then shelled. In the semi-wet processing method, coffee beans with hulls were inoculated with the yeasts. After that, they were sun-dried for two days until the moisture content reached 15.0 to 24.5%. Their hulls were then shelled and further sun-dried until the moisture content reached 9.6 to 11.8%. The moisture content of coffee beans was determined using Moisture Meter DELMHORST Model G-7. The moisture content of coffee beans processed using wet and semi-wet methods is presented in Table 1.

Table 1 Moisture contents of coffee beans processed using wet and semi-wet methods

No	Inoculation treatment	Moisture content (%)		
		Wet method	Semi-wet method	
			First drying	Second drying
1	Control (without inoculation)	9.7	15.0	10.0
2	Commercial lactic acid bacteria	9.7	22.1	9.9
3	*I. orientalis* BIO 211287	9.5	17.5	9.7
4	*I. orientalis* BIO 211288	9.4	21.7	10.0
5	*I. orientalis* BIO 211289	10.0	18.1	10.9
6	*Aspergillus ochraceus* BIO 37310	9.4	24.5	10.7
7	*A. ochraceus* BIO 37310-*I. orientalis* BIO 211287	9.6	21.4	11.8
8	*A. ochraceus* BIO 37310-*I. orientalis* BIO 211288	9.2	21.2	11.0
9	*A. ochraceus* BIO 37310-*I. orientalis* BIO 211289	9.2	23.2	11.0

Isolation of the yeasts and *A. ochraceus* BIO 37310 from each treatment (including the control) was determined using the dilution method, followed by the pour plate method in Yeast Malt Extract Agar (YMEA) and PDA, respectively. Their population was determined based on the number of fungal colony on YMEA and/or PDA in a certain dilution factor.

Mechanism of Antagonisms between the Potential Yeast Isolates and *A. ochraceus* BIO 37310

The direct opposition method was applied to Potato Dextrose Agar (PDA) on a Petri dish (9 cm in diameter) to obtain information about the mechanism of antagonism between the most potential yeast isolate and *A. ochraceus* BIO 37310 (Skidmore & Dickinson 1976) (Fig. 1).

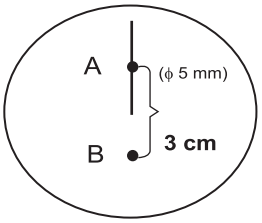

Figure 1 Antagonism test between the most potential yeast isolate and *A. ochraceus* BIO 37310
Notes: A = *A. ochraceus* BIO 37310; B = Yeast isolate.

A. ochraceus BIO 37310 (5 mm in diameter) was placed in PDA on a Petri dish (9 cm in diameter) three days after yeast inoculation on the same plate with a distance of 3 cm. Three replicates (= three Petri dishes) were used for each treatment. All Petri dishes were incubated at room temperature (± 28 °C) for seven days. The observation on the mechanism of antagonism was conducted macroscopically, i.e., by observing the types of interaction between the yeast isolate and *A. ochraceus* BIO 37310 (Wheeler & Hocking 1993).

Cupping Test

Cupping test of the samples was conducted based on the Standard Cupping Protocol issued by Coffee Quality Institute (CQI) and Specialty Coffee Association of America (SCAA) (2015). Each panelist has a certificate from a CQI Q grader. Cupping test was conducted at the Coffee Laboratory of PT Kemenady Industri Mandiri, Bogor.

Statistical Analyses

The data of biocontrol assay of *A. ochraceus* BIO 37310 using three yeast isolates *in-vivo* were analyzed using a completely randomized factorial design with two factors, i.e., method of coffee bean processing and yeast inoculation, including the control.

RESULTS AND DISCUSSION

Screening for Antagonistic Yeasts against *A. ochraceus* BIO 37310

The diameter of *A. ochraceus* BIO 37310 and each yeast isolate in antagonism test is presented in Table 2. *Endomyces decipiens* BIO 131215, *I. orientalis* BIO 211286, BIO 211287, BIO 211288, and BIO 211289 could totally inhibit the growth of *A. ochraceus* BIO 37310. Controls of *I. orientalis* BIO 211288 and *A. ochraceus* BIO 37310, and antagonisms test between *I. orientalis* BIO 211288 and *A. ochraceus* BIO 37310 are presented in Figure 2.

Table 2 The growth (colony diameter) of *A. ochraceus* BIO 37310 and yeast isolates in the antagonism test between *A. ochraceus* BIO 37310 and 22 yeast isolates

No.	A. *ochraceus* BIO 37310 vs yeast isolates	Colony diameter (mm)	
		A. ochraceus	Yeast
1.	Ao BIO 37310-yeast isolate KA	34	0
2.	Ao BIO 37310-yeast isolate KA2	26.75	0
3.	Ao BIO 37310-yeast isolate KB	31.5	0
4.	Ao BIO 37310-yeast isolate KB2	17.25	3.25
5.	Ao BIO 37310-yeast isolate KC	21.75	13.25
6.	Ao BIO 37310-yeast isolate KD	37.25	0
7.	Ao BIO 37310-*Endomyces decipiens* BIO 211215	0	36.75
8.	Ao BIO 37310-*E. fibuliger* BIO 211216	28.5	0
9.	Ao BIO 37310-*E. fibuliger* BIO 211217	Contamination	47.5
10.	Ao BIO 37310-*E. fibuliger* BIO 211218	34.5	0
11.	Ao BIO 37310-*E. fibuliger* BIO 211219	42.5	0
12.	Ao BIO 37310-*E. fibuliger* BIO 211220	10.75	10
13.	Ao BIO 37310-*Issatchenkia orientalis* BIO 211285	18.75	36.25
14.	Ao BIO 37310-*I. orientalis* BIO 211286	0	22.5
15.	Ao BIO 37310-*I. orientalis* BIO 211287	0	46.75
16.	Ao BIO 37310- *I. orientalis* BIO 211288	0	63
17.	Ao BIO 37310- *I. orientalis* BIO 211289	0	42.75
18.	Ao BIO 37310- *I. orientalis* BIO 211290	21.5	6.75
19.	Ao BIO 37310- *I. orientalis* BIO 211291	37	0
20.	Ao BIO 37310-*Saccharomyces cerevisiae* BIO 211363	33.25	0
21.	Ao BIO 37310-*S. cerevisiae* BIO 211364	Contamination	Contamination
22.	Ao BIO 37310-*S. cerevisiae* BIO 211 365	37.25	0

Note: Ao = *Aspergillus ochraceus.*

(1a) (1b) (2)

Figure 2 Antagonism test between yeast isolates and *A. ochraceus* BIO 37310 using well (dip) method in PDA containing 15% Arabica coffee beans extract after seven days of incubation at temperature 28 ± 2 °C
Notes: (1a) = *A. ochraceus* BIO 37310 vs *Issatchenkia orientalis* BIO 211288; (1b) = control of *A. ochraceus* BIO 37310; (2) = control *I. orientalis* BIO 211288; scale bar = 2 cm.

According to Pereira *et al.* (2015), microorganisms (especially yeasts and lactic acid bacteria) may also contribute to a beverage's sensory characteristics and other qualities in the fermentation process. Yeasts are among the most frequently isolated microorganisms found in fermented coffee beans.

Pichia anomala CCMA0148 and *Saccharomyces cerevisiae* CCMA0159 inhibited fungal growth with a high inhibitory rate. It means that the yeasts can be a potential biocontrol agent in controlled conditions (de Souza *et al.* 2017).

Biocontrol Agent of *A. ochraceus* BIO 37310 using Yeasts *In-Vivo*

Issatchenkia orientalis (BIO 211287, BIO 211288, and BIO 211289) were used in the biocontrol test of *A. ochraceus* BIO 37310 *in-vivo* because the yeasts could inhibit the growth of *A. ochraceus* BIO 37310 *in-vitro*.

Aspergillus ochraceus BIO 37310 did not grow in coffee beans in all treatments, either in wet or semi-wet processing methods and yeasts inoculation, including the coffee beans, were inoculated with *A. ochraceus* BIO 37310 and commercial lactic acid bacteria. It was assumed that the treatments could decrease the concentration of *A. ochraceus* BIO 37310 conidia (lower than 5 x 10^4 conidia/mL). Interaction between processing methods and yeast or *A. ochraceus* BIO 37310 inoculation had a very significant effect on yeast population (Table 3).

Issatchenkia orientalis (BIO 211287 and BIO 211288) grew well in coffee beans processed using a semi-wet method. The highest yeast population was found in coffee beans processed using the semi-wet method and inoculated with *I. orientalis* BIO 211288 (46,222 ± 9,576 cfu/g), and it was not significantly different from that of the coffee beans inoculated with *I. orientalis* BIO 211287 (36,333 ± 14,000 cfu/g) (Table 4).

Issatchenkia orientalis (BIO 211287, BIO 211288, and BIO 211289) grew well in coffee beans processed using wet and semi-wet methods inoculated with *A. ochraceus* BIO 37310. This result indicates that the yeast isolates inhibited the growth of *A. ochraceus* BIO 37310. The population of yeast isolate BIO 211287 in coffee beans processed using the semi-wet method (32,444 ± 6,159 cfu/g) inoculated with *A. ochraceus* BIO 37310 and *I. orientalis* BIO 211287 was higher than that in coffee beans processed using the wet method (12,445 ± 13,184 cfu/g). The yeast population of coffee beans inoculated using commercial lactic acid bacteria and without yeast, processed either using wet or semi-wet methods, was relatively low.

Table 3 Analysis of variance on the effect of processing method of three yeast isolates and/or *A. ochraceus* BIO 37310 inoculation on yeast population

Source of variance	df	SS	MS	F-value
Processed method (A)	1	1831616398	1831616398	17.06**
Three yeast isolates and/or *A. ochraceus* BIO 37310 treatment (B)	7	4736862286	676694612	6.30**
AB	7	1762700395	251814342	2.35**
Error	32	3435612218	107362882	

Notes: * = Significantly different at 95% confidence level; ** Very significantly different at 99% confidence level.

Table 4 Yeast population in coffee beans processed using wet and semi-wet methods and inoculated with either three yeast isolates or *A. ochraceus* BIO 37310

Inoculation treatment and control	Processing method	
	Wet (cfu/g)	Semi-wet (cfu/g)
Control (without inoculation)	2,411 ± 548 ag	71 ± 31 a
Inoculated with commercial lactic acid bacteria	2,422 ± 1,496 ag	4,267 ± 416 afg
Inoculated with *I. orientalis* BIO 211287	12,333 ± 6,333 adefg	36,333 ± 14,000 bh
Inoculated with *I. orientalis* BIO 211288	13,667 ± 9,207 acdefg	46,222 ± 9,576 h
Inoculated with *I. orientalis* BIO 211289	6,889 ± 1,678 aefg	21,667 ± 2,729 bcdefg
Inoculated with *A. ochraceus* vs *I. orientalis* BIO 211287	12,445 ±13,184 adefg	32,444 ± 6,159 bch
Inoculated with *A. ochraceus* vs *I. orientalis* BIO 211288	26,556 ± 17,024 bcde	23,444 ± 2,365 bcdef
Inoculated with *A. ochraceus* vs *I. orientalis* BIO 211289	17,778 ± 7,691 abcdefg	28,889 ± 26,943 bcdh

Notes: Numbers followed by the same letter in the same row do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

According to Velmourougane *et al.* (2011), dip treatment containing a yeast suspension (*Saccharomyces cerevisiae*) was used in coffee postharvest processing and resulted in a significant reduction of total mold incidence (*Aspergillus niger*, *A. ochraceus*) and ochratoxin A contamination without affecting the cup quality. According to Masoud *et al.* (2005), three yeasts, i.e., *Pichia anomala, P. kluyveri,* and *Hanseniaspora uvarum*, inhibited fungal growth. The effect of their combination was more effective than individual yeast species for inhibiting fungal growth. *Aspergillus ochraceus* was inhibited by the three yeasts with the highest inhibitory rate (53%).

Mechanisms of Antagonism between the Most Potential Yeast Isolates and *A. ochraceus* BIO 37310

Three potential *I. orientalis* isolates (BIO 211287, BIO 211288, and BIO 211289) were used to study the mechanisms of antagonism test against *A. ochraceus* BIO 37310 using direct opposition method.

Interaction between *I. orientalis* (BIO 211287, BIO 211288, and BIO 211289) and *A. ochraceus* BIO 37310 resulted in E-type interaction, i.e., the fungus was not able to grow anymore, while yeast isolates grew further. Based on visual observation, the colony of the three yeast isolates and *A. ochraceus* BIO 37310 had a mutual contact without inhibitory zone (Fig. 3). It was assumed that space and nutrient competitions were the mechanism of antagonism between the yeasts and the fungus. It was also proven that the yeast isolates colonized rapidly in well (dip) method, using the space and nutrient in Potato Dextrose Agar (PDA) containing 15% Arabica coffee beans extract. Therefore, the yeast isolates inhibited the growth of *A. ochraceus* BIO 37310. Other mechanisms of antagonism were chitinase production, adherence to fungal cell wall, peroxidase activity, endurance induction (El Gouth *et al.* 2003), and secretion production that inhibited pathogen growth (Guetsky *et al.* 2002).

Cupping Test

According to the Specialty Coffee Association of America (SCAA), specialty coffee beans are not accepted if the cupping score is lower than 80.

In general, coffee was not consumed because of its nutritional value but because of its flavor and physiological influences that cause people to stay awake, add freshness, reduce fatigue, and create a more excited feeling (Atmawinata 2002). Saepudin (2005) reported that the value of coffee beans was not only determined by physical quality but also by flavor. Therefore, cupping test is one of the methods to determine the quality of coffee in importing countries.

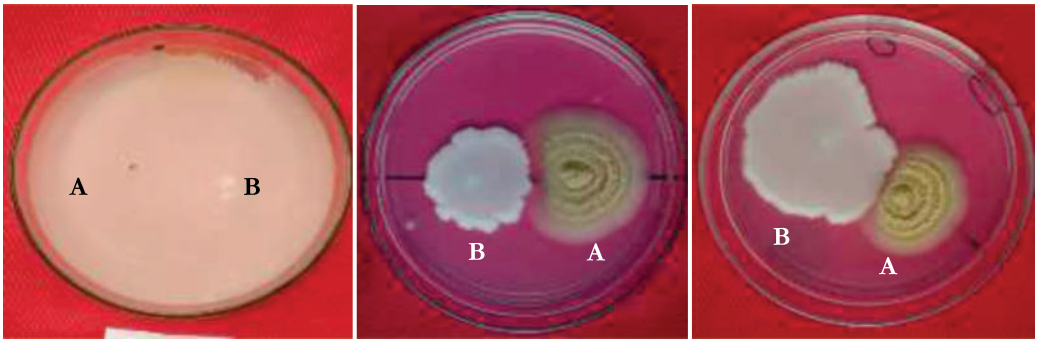

Figure 3 Mechanisms of antagonism between three *I. orientalis* isolates: (a) BIO 211287; (b) BIO 211288; and (c) BIO 211289) and *A. ochraceus* BIO 37310 which showed E-type interaction in PDA after seven days of incubation at temperature 28 ± 2 °C
Notes: A = *A. ochraceus* BIO 37310; B = *I. orientalis* isolate; scale bar = 2 cm.

Table 5 Cupping scores of coffee beans inoculated with *Issatchenkia orientalis*, commercial lactic acid bacteria, and control (not inoculated with yeast)

Attribute	Score				
	I. orientalis BIO 211287	*I. orientalis* BIO 211288	*I. orientalis* BIO 211289	Commercial lactic acid bacteria	Control (not inoculated with *I. orientalis*)
Fragrance	7.5	7.5	7.5	7.5	7.5
Flavor	7.5	7.5	7.5	7.25	7.5
Aftertaste	7.75	7.5	7.5	7.25	7.25
Acidity	7.5	7.5	7.5	7.5	7.5
Body	7.5	7.5	7.5	7.5	7.5
Balance	7.5	7.5	7.5	7.5	7.5
Uniformity	10	10	10	10	10
Clean cup	10	10	10	10	10
Sweetness	10	10	10	10	10
Overall	7.5	7.5	7.5	7.5	7.5
Total score	82.75	82.50	82.50	82.00	82.25
Defect	0	0	0	0	0
Final score	82.75	82.50	82.50	82.00	82.25

Laboratory of cupping test: Coffee Laboratory of PT Kemenady Industri Mandiri, Bogor
Notes on scores: 0 = not present; 1 = unacceptable; 2 = very poor; 3 = poor; 4 = fair; 5 = average; 6 = good; 7 = very good; 8 = excellent; 9 = outstanding; 10 = exceptional.

Qualification of coffee beans is based on a final score which is divided into four categories, i.e., outstanding (90 - 100), excellent (85 - 89.99), very good (80 - 84.99), and below specialty quality (below 80). Coffee beans which are marked as outstanding, excellent, and very good with a final score of 80 - 100 belong to specialty qualification. Meanwhile, coffee beans which are marked as below specialty category do not belong to specialty qualification but can still be consumed. According to panelists, coffee beans could not be consumed if the final score was ≤ 30 (SCAA 2015).

The flavor of coffee beans which were inoculated with the yeasts was still above the score limit for specialty qualification, i.e., ≥ 80 (Table 5). The total score of coffee beans inoculated using three potential yeasts to control the growth of *A. ochraceus* BIO 37310 was higher than those which were uninoculated and inoculated by commercial lactic acid bacteria. The highest total score was obtained by coffee beans inoculated with *I. orientalis* BIO 211287 (82.75). This result was not significantly different to that of the biocontrol agent test of *A. ochraceus* BIO 37310 using two yeast isolates (*I.orientalis* BIO 211288 and BIO 211289). It means that the quality of coffee beans inoculated with *I. orientalis* BIO 211287, BIO 211288, and BIO 211289 were better than coffee beans inoculated with commercial lactic acid bacteria and control. The population of the yeasts was higher than that without yeast and inoculated with commercial lactic acid bacteria (Table 4).

CONCLUSION

This research provides an opportunity of using three yeast isolates of *Issatchenkia orientalis* (BIO 211287, BIO 211288, and BIO 211289) as biocontrol agents of OTA-producing fungus (*Aspergillus ochraceus* BIO 37310) and to increase the sensorial quality of coffee beverages. *In-vitro,* three yeast isolates of *I. orientalis* were able to totally inhibit the growth of the fungus. *In-vivo,* the yeast isolates were able to grow in coffee beans inoculated with the fungus, when either the wet or semi-wet method was used to process the coffee beans. Yeast population was higher in coffee beans processed using the semi-wet method. The total cupping score of coffee beans inoculated with the yeasts was higher than that of those uninoculated and inoculated with commercial lactic acid bacteria. The result of this research shows that *I. orientalis* BIO 211287, BIO 211288, and BIO 211289 could be used as biocontrol agents of *A. ochraceus* BIO 37310 and increase the sensorial quality of coffee beverage.

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