The Test of Saccharomyces sp. Potential Filtrate to Inhibit The Growth of Aspergillus flavus FNCC6109 Broiler Chicken Concentrate Feed Model

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Abstract: The test of *Saccharomyces* sp. culture filtrate potential aims to determine the ability of *Saccharomyces* sp. isolates that was obtained on Bali cattle *swap saliva* by *in vitro* and *in vivo* tests on FNCC6109 *Aspergillus flavus* in broiler chicken concentrate feed model. The highest inhibitory ability on *A. flavus* FNCC6109 growth in vitro with experimental method was conducted in *Saccharomyces* sp. filtrate culture. The in vivo study used 24 experimental units divided into 8 treatment groups with 3 replicates respectively, i.e. A: Concentrate without *A. flavus* FNCC6109 and without Sc.I culture filtrate; B: Concentrate + 15 mL of sterile water; C: Concentrate + A.flavus FNCC6109; D: Concentrate + *A.flavus* FNCC6109 + 10% Sc.I; E: Concentrate + *A.flavus* FNCC6109 + 20% Sc.I; F: Concentrate + *A.flavus* FNCC6109 + 30% Sc.I; G: Concentrate + *A.flavus* FNCC6109 + 40% Sc.I; H: Concentrate + *A.flavus* FNCC6109 + 50% Sc.I. with a 15 days of storage period. The quantitative results data was analyzed using ANOVA assay and followed by *Duncan* test. The filtrate culture had been incubated for 48 hours at 62.6%, therefore it could be used in in vivo testing. The addition of *Saccharomyces* sp.I culture filtrate concentrate by 40% and 50% was able to inhibit the population of *A. flavus* FNCC6109 by 97% in broiler chicken concentrate feed model. The results showed a significant difference ($P \leq 0.05$), which means that *Saccharomyces* sp.I culture filtrate with the concentration of 40% and 50% in broiler chicken concentrate feed model had the highest inhibition on the total population of *A. flavus* FNCC6109.

1 INTRODUCTION

Livestock business in Indonesia is dominated by local farms with quite large production output (Subandriyo, 2006). Lack of feed availability can lead to the decrease of production, decreased health status and bad effects on livestock reproduction (Saptahidayat, 2005).

According to Sudarmono and Sugeng (2008), in general animal feed ingredients are classified into three types, namely forage feed, concentrate feed and additional feed. According to Kartadisastra (1997), concentrate feed is a staple food made from a mixture of several sources of nutrients such as energy, protein, vitamins and minerals. Feed quality is not only determined from the nutrient value composition of the feed, but it also must be free of contamination such as aflatoxin that has the potential to contaminate fodder (Rachmawati, 2005).

Aflatoxin that contaminates the concentrate feed and its processed ingredients is produced by *Aspergillus flavus*. The optimum condition of this mold in producing aflatoxin is at the temperature of 25-300C with relative humidity 85% and water content 15-30% (Dwidjoseputro, 1989). According to Rachmawati (2004), maize is the basic ingredient of feed and used most up to 50-60% in poultry rations.

Application of Saccharomyces sp. as a biocontrol agent is one of the efforts to prevent the pathogen growth. Further research conducted by El-Sayed and Eman, (2011) mentioned the use of yeast as a biocontrol agent in controlling leaf disease in sugar beet plant with the application of 5 types of yeast and fungicide significantly reduced leaf infection in sugar beet plant compared with control.

Effort to suppress the growth of A. flavus FNCC6109 is still important. Therefore, it is

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necessary to study the Saccharomyces sp. culture filtrate potential to be used in the field of animal husbandry to control A.flavus contamination in concentrate feed as an effort to increase livestock productivity.

2 MATERIAL AND METHODS

2.1 Preparation of *Saccharomyces sp.* Culture Filtrate in Broth Media

The isolated yeast successfully isolated from Bali cattle (data was not shown) was grown on Yeast Extract Peptone Dextrose (YEPD) Broth media by taking 1 dose inoculated on 3 Erlenmeyer containing 25 mL of YEPD Broth media. Each Erlemenyer containing media and isolates was incubated consecutively at room temperature for 24 hours; 48 hours and 72 hours

2.2 Inhibitory Test of *Saccharomyces sp.* Filtrate Culture on *Aspergillus flavus* FNCC6109

Inhibitory test of *Saccharomyces sp.* filtrate culture was conducted experimentally by preparing 3 sterile Petri dishes, each Petri dish was deposited with 1 mL of Saccharomyces sp. culture filtrate that had been incubated for 24 hours; 48 hours and 72 hours, after that it was poured with 15 mL of PDA media and then shaken simultaneously to obtain a homogeneous mixture. After the culture mixture of the filtrate and media solidified, then right in the middle of the Petri dish a piece of *A. flavus* colony with a diameter of 0.5 cm was placed. As for the control, sterile Petri dish filled with 1 mL of sterile water and 15 mL of PDA media was prepared, as well as A. flavus with a diameter of 0.5 cm. All the treated Petri dishes were incubated at room temperature for 7 days and repeated 5 times

2.3 Effects of *Saccharomyces sp.* Filtrate Culture on *Aspergillus flavus* FNCC6109 Population in Broiler Chicken Concentrate Feed Model

Effects of *Saccharomyces sp.* culture filtrate on *A. flavus* FNCC6109 population in broiler chicken concentrate feed model was obtained by Completely Randomized Design (RAL) with 8 treatment types and 3 replications. *Saccharomyces sp.* isolates used in in vivo testing was the ones with the highest inhibitory ability in the previous test (in vitro). Before the formulation was done, the feed ingredient was treated in autoclave first. Treatment to the concentrate feed model included:

A: Concentrate without *A. flavus* FNCC6109 and without Sc.I culture filtrate; B: Concentrate + 15 mL of sterile water; C: Concentrate + *A.flavus* FNCC6109; D: Concentrate + *A.flavus* FNCC6109 + 10% Sc.I; E: Concentrate + *A.flavus* FNCC6109 + 20% Sc.I; F: Concentrate + *A.flavus* FNCC6109 + 30% Sc.I; G: Concentrate + *A.flavus* FNCC6109 + 40% Sc.I; H: Concentrate + *A.flavus* FNCC6109 + 50% Sc.I. After treatment, all of the feed was dried in an oven with a temperature of 400C for 48 hours. Concentrate feed was then stored for 15 days at room temperature. Observation of total *A. flavus* FNCC6109 population was determined by using plating method with dilution series (Nester et al., 2007).

3 RESULT

3.1 The *Saccharomyces sp.* Filtrate Culture Inhibitory Potential to the Growth of *Aspergillus flavus* FNCC6109 in Vitro

From in vitro test, the results obtained was the percentage of *Saccharomyces sp.* culture filtrate inhibitory power where the highest was 63.6 ± 2.07% by *Saccharomyces sp.*I culture filtrate isolates with an incubation period of 48 hours. When compared to *Saccharomyces sp*.II culture filtrate isolates, the highest inhibition percentage occurred at incubation period for 24 hours of 60.8 ± 8.43%. However, when compared with the control treatment of *A. flavus* FNCC6109 diameter that grew on PDA media and in incubation for 7 days, it reached 4.00 cm (data was not shown).

The data shown in Table 1 shows that the treatment of *Saccharomyces sp*.I culture filtrate with 48-hours incubation period used in this study had the highest inhibitory ability so that it can proceed to the in vivo testing stage by testing several concentrations of the *Saccharomyces sp*.I filtrate culture added to the broiler chicken feed concentrate model in inhibiting the growth of A. flavus FNCC6109.

Table 1: Percentage of *Saccharomyces* sp. filtrate inhibition at different incubation periods to the growth of A. flavus FNCC6109.

Saccharomyces sp. Culture Filtrate Isolate	Incubation Period (Hour)	Diameter of *A. flavus* colony FNCC6109 (cm)					Mean (cm)	Inhibition (%)
		1	2	3	4	5		
KFS I	24	1.5	1.4	0.9	1.15	1.25	1.24±0.23	52.2±2.15
	48	1.1	1.0	1.15	1.15	1,2	1.12±0.07	63.6±2.07
	72	1.4	1.25	1.45	1.1	1.2	1.28±0.14	56.2±5.67
KFS II	24	1.5	1.1	1.45	1.7	1.2	1.39±0.24	60.8±8.43
	48	1.9	2.3	2.05	1.2	1.5	1.79±0.43	53.0±10.3
	72	1.5	1.7	2.2	2.35	1.45	1.84±0.41	52.0±8.52

Description: KFS I: *Saccharomyces* spI Filtrate
KFS II: *Saccharomyces* sp.I Filtrate

3.2 *Aspergillus flavus* FNCC6109 Populationin Chicken Broiler Concentrate Feed Model Added with Isolate Filtrate *Saccharomyces sp.* I

The analysis result of total *Aspergillus flavus* FNCC6109 population on broiler chicken feed concentrate model showed the decrease in the total population of *A. flavus* FNCC6109 after given Saccharomyces sp. culture filtrate I with various concentration. Differences in *A. flavus* FNCC6109 population before and after storage for 15 days were able to maintain the quality of concentrate feed. The highest population of *A. flavus* FNCC6109 was found in concentrate feed which only added A. flavus FNCC6109 suspension at 29x105 CFU/g before storage and 66.2x105 CFU/g after storage. The lowest population of *A. flavus* FNCC6109 was found in the concentrate feed model with the addition of 50% (15mL/25gr) concentration of Saccharomyces sp.I culture filtrate by 1.4x105 CFU/g.

Table 2: Total population of *Aspergillus flavus* FNCC6109 in broiler chicken feed concentrate model added by *Saccharomyces sp*.I filtrate before and after storage period.

Treatment	Total *A.flavus* FNCC6109 (CFU/g) population		% Increase of *A.flavus* FNCC6109
	Population before storage (T_0)	Population after storage (T_{15})	
A	(0.00)	$(0.00)^{a}$±0.00	0
B	(0.00)	$(0.00)^{a}$±0.00	0
C	$29x10^5$	$66.2x10^{5d}$±0.151	56
D	$3.0x10^5$	$4.7x10^{5b}$±0.693	36
E	$2.0x10^5$	$3.0x10^{5b}$±0.714	33
F	$2.0x10^5$	$3.0x10^{5bc}$±0.051	33
G	$1.1x10^5$	$1.6x10^{5c}$±0.122	31
H	$1.0x10^5$	$1.4x10^{5c}$±0.020	28

Table 2 shows the effect of the addition of Saccharomyces sp.I culture filtrate to the total population of A. flavus FNCC6109 after concentrate feed model was stored for 15 days. The total population of A. flavus FNCC6109 prior to storage period had shown a decrease in some concentrate feed models that had been added Saccharomyces sp.I culture filtrate. The results of statistical analysis using Duncan Multiple Range Test (DMRT) showed a significantly different mean value ($P \leq 0.05$) between controls (A and C) with the concentration of each treatment (D, E, F, G, and H).

In the concentrate feed model without the addition of *A. flavus* FNCC6109 suspension and *Saccharomyces sp*.I culture filtrate before and after storage period, the growth of *A. flavus* FNCC6109 after analysis with dilution method was not found. This could be due to the sterilization process on the concentrate feed model that was running well so that there was no contamination from other microorganisms. The population of *A. flavus* FNCC6109 contained in the concentrate feed model was 66.2x105 CFU/g with rate of increase reached 56%. While the lowest population of *A. flavus* FNCC6109 was found in the concentrate feed model that was added with *Saccharomyces sp*.I culture filtrate with 50% concentration of 1.4x105 CFU/g with the increase only 28%.

4 DISCUSSION

The small diameter size of *A. flavus* that was tested in vitro by Saccharomyces sp. culture filtrate proved the effect of an enzyme or other compound excreted by Saccharomyces sp. culture. According to the research conducted by Chan and Tian (2005) in vitro, by using modification method on Saccharomyces sp. ability in lysing the cell wall of *A. parasiticus*, there was a direct interaction of Saccharomyces sp. cells on the hyphae of A. parasiticus. It was allegedly due to β-gluconase enzyme activity produced by Saccharomyces sp. Furthermore, Albers et al. (1996) mentioned that yeast culture filtrate is capable to produce several types of enzymes and organic acids such as ethanol, glycerol, acetic acid, pyruvic acid, succinic acid, á-ketoglutarate and fumaric acid. In addition to the inhibitory ability possessed by yeast isolates, the role of lactic acid bacteria such as *Lactobacillus plantarum* is able to inhibit spore germination from *A. flavus* due to pH changes in fermentation media and nutrient competition (Xu et al., 2002).

The ability of *Saccharomyces sp*.I culture filtrate to inhibit the growth of *A. flavus* FNCC6109 in the concentrate feed model was suspected to occur due to the nutrient competition and culture ability in producing primary metabolite. A research from Dharmaputra et al. (2003) mentioned that mold has a faster growth ability compared with *A.flavus* that has the potential to control *A. flavus* attack on peanut seeds. Based on these results, the percentage of inhibition to the growth of *A. flavus* FNCC6109 from the addition of Saccharomyces sp.I culture filtrate with concentration of 40% and 50% during storage period had percentage of inhibition equal to 97%. The results were consistent with a study conducted by Darmayasa (2015) stating that the administration of *Trichoderma asperellum* TKD filtrate with a concentration of 9g/100g could inhibit the growth of *A. flavus* FNCC6109 in the concentrate feed model of 74.93% with 30 days of storage period. Raharjanti (2006) also mentioned that the culture filtrate of M. rouxii and Saccharomyces sp. was able to inhibit the growth and affected the morphological structure of *A. parasiticus*. However, if compared with the *M. rouxii* culture filtrate, the inhibitory ability of *Saccharomyces sp*. culture filtrate was much higher as it reached 98.1%.

5 CONCLUSIONS

Based on the research results, it can be concluded that between 2 isolates obtained from swap saliva of Bali cattle, the ability of *Saccharomyces* sp.I culture filtrate used in this study generally has positive correlation between in vitro and in vivo testing in inhibiting the growth of *A. flavus* FNCC6109.

The *Saccharomyces sp*.I culture filtrate potential in inhibiting the growth of *A.flavus* FNCC6109 in the concentrate feed model provides an effect in decreasing the number of *A. flavus* FNCC6109 after 15 days of storage.

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