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<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 25-30OC 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 Enman, (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
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 (YPED) Broth media by taking 1 dose inoculated on 3 Erlenmeyer containing 25 mL of YEPD Broth media. Each Erlenmeyer 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 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 40°C 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.

<table>
<thead>
<tr>
<th>Saccharomyces sp. Culture Filtrate Isolate</th>
<th>Incubation Period (Hour)</th>
<th>Diameter of A. flavus colony FNCC6109 (cm)</th>
<th>Mean (cm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>KFS I</td>
<td>24</td>
<td>1.5</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>1.1</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>1.4</td>
<td>1.25</td>
<td>1.45</td>
</tr>
<tr>
<td>KFS II</td>
<td>24</td>
<td>1.5</td>
<td>1.1</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>1.9</td>
<td>2.1</td>
<td>2.05</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>1.5</td>
<td>1.7</td>
<td>2.2</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total A. flavus FNCC6109 (CFU/g) population</th>
<th>% Increase of A. flavus FNCC6109</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Population before storage (T0)</td>
<td>Population after storage (T10)</td>
</tr>
<tr>
<td>A</td>
<td>(0.00)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>B</td>
<td>(0.00)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>C</td>
<td>2.9×10⁵</td>
<td>6.8×10⁵</td>
</tr>
<tr>
<td>D</td>
<td>3.0×10⁵</td>
<td>4.1×10⁵</td>
</tr>
<tr>
<td>E</td>
<td>2.0×10⁵</td>
<td>3.0×10⁵</td>
</tr>
<tr>
<td>F</td>
<td>2.0×10⁵</td>
<td>3.8×10⁵</td>
</tr>
<tr>
<td>G</td>
<td>1.4×10⁵</td>
<td>1.6×10⁵</td>
</tr>
<tr>
<td>H</td>
<td>1.0×10⁵</td>
<td>1.4×10⁵</td>
</tr>
</tbody>
</table>

3.2 Aspergillus flavus FNCC6109 Population in 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 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-glucanase 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, α-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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REFERENCES


