Growth analysis of *Microsporum canis* using husk rice (*Oryza Sativa* L. CV. Ciherang) as a replacement for media Sabouroud Dextrose Agar (SDA)

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Abstract. Rice husk is a hard layer covering caryopsis which consists of two parts called the lemma and palea which are interconnected in the milling process. Rice husk is categorized as biomass that can be used for variety of needs such as raw materials and animal feed. Rice husk containing sources of carbohydrates and proteins which are the factors those support the growth of fungi. Sabouroud Dextrose Agar (SDA) is medium that could be used for the growth of fungi. Experimental research had been carried out by inoculating *Microsporum canis* in alternative media such as rice husk. This study aimed to compare the growth of *Microsporum canis* in alternative media and control media. It has been done by varying concentrations of rice husk media into 4.5%, 5%, and 5.5%. Observations done in 14 days, at room temperature with macroscopic examination each day by measuring the colony diameter using calipers. Microscopic examinations measure the length of macroconidia and *Microsporum canis* hyphae using microscope equipped with microoculary lens by staining using Lactophenol Cotton Blue (LPCB). Observations processed statistically using one-way ANOVA. From the result of the statistical test was concluded that rice husk powder concentration of 5.5% is the optimum concentration for the growth of *Microsporum canis* and can be used as a replacement media for Sabouroud Dextrose Agar (SDA).

1. Introduction

*Microsporum canis* is a fungus that causes dermatophytosis primarily attacks animals and occasionally human [1-6]. Usually commercial media of fungi include SDA which contains various nutrients for the growth of fungi such as carbohydrates and proteins. Usually composition of SDA is set to fulfil basic needs of fungi as they live in natural habitat. But sometimes by using SDA media, some types of fungi cannot be isolated or can be isolated but do not grow well. To overcome the ability limitation of SDA in fungi isolation, modification is needed to speed up a diagnosis. Unfortunately, synthetically modification is very complicated, needs accuracy, long time, and expensive chemical material [1-3, 5, 7-11].

Rice husk is a by-product of rice by being dried through grinding or pounding and then separated. Rice husk then can be used as fungi growing media [2-6,10-14]. Actually, rice husk contains water (9.02%), crude protein (3.03%), fat (1.18%), and carbohydrate (33.71%), while chemical compositions of rice husk are carbon, hydrogen, oxygen, and silica. [2-3,6,8,10-12,15].

In previous research, media alteration using rice husk as a substitute had been experimented for particular type of fungi and they grew well as well as SDA medium. Based on those experiments,
researchers were interested to make experiment rice husk as alternative growing media with optimum growth. To search optimum growth, rice husk concentrations tested were 4.5%, 5%, and 5.5%.

2. Methods
Research conducted in March 2016, located at STABA Laboratory. Microsporum canis taken from the Faculty of Medicine UI Jakarta, Indonesia. Rice husk taken from milling factory at Ciherang then dried at Padasuka Atas Bandung area. Research method was experimental. Tools used in this study were autoclave, stirring rod, petri dish, Erlenmeyer, chemical glass, hot plate, incubator, object glass, cover glass, asbestos cases, spiritus burner, microscope, weighing tool, Ose needle, horn spoon, and water bath. Materials used agar-agar rod, aquadest, spiritus, alcohol 70%, Antibiotics Chloramfenicol, pure Microsporum canis culture, rice husk that had been mashed, SDA (Sabouraud Dextrosa Agar), and LPCB.

![Figure 1. Growth of Microsporum canis at rice husk media with concentration of 4.5% (Arrow sign).](image1)

![Figure 2. Growth of Microsporum canis at rice husk media with concentration of 5% (Arrow sign).](image2)

![Figure 3. Growth of Microsporum canis at rice husk media with concentration of 5.5% (Arrow sign).](image3)

![Figure 4. Growth of Microsporum canis at SDA control media (Arrow sign).](image4)
Figure 5. Hyphae length of *Microsporum canis* at 5.5% concentration of rice husk (Arrow sign).

Figure 6. Macroconidia length of *Microsporum canis* at 5.5% concentration of rice husk (Arrow sign).

Figure 7. Hyphae length of *Microsporum canis* at SDA control media (Arrow sign).

Figure 8. Macroconidia length of *Microsporum canis* at SDA control media (Arrow sign).

Comparison on the measurement of colony diameter of *Microsporum canis* using 4.5%, 5%, 5.5% concentration, and SDA control media can be seen at Figure 8.

Figure 9. Colony diameter growth comparison between rice husk media with concentration of 4.5%, 5%, 5.5% and at SDA control media.
3. Result and Discussion

This study was an experimental test that aims to see the growth of *Microsporum canis* grown on alternative media using rice husk powder and compared with natural resources (figure 1,2,3,4,5,6,7,8). In addition, to determine significant differences in diameter of colony and macroconidia on a variety of alternative media of rice husk concentration respectively 4.5%, 5%, and 5.5%, with the SDA as a control, then measuring the diameter and the length of the hypha embedded in each medium (figure 1,2,3). In this study, *Microsporum canis* observations were grown on SDA control media and alternative media by adding antibiotic chloramphenol for preventing bacterial growth, pH 5.6, and *Microsporum canis*
inoculated on the media by single dot, and incubated at room temperature. Based on the measurements of hyphae and microconidia Microsporum canis using an ocular micrometre with μm units, the mean diameter of hyphae on alternative media and SDA control media obtained results that there were no significant differences (figure 1,2,3). In media of SDA and alternative media of rice husk Microsporum canis consuming 1-3 days as adaptation phase (phase lag). In phase adaptation, this fungus was in the stage of adjusting to new environment. This condition could occur due to changing the environment on standard media and alternative media. In this phase, the cells formed enzymes to break down the substrate. Lag phase in microorganism has a varieties type depending on media composition, temperature, pH and physiological properties of a microorganism. After the adaptation phase on the sixth day of the fungi had the acceleration phase, the starting phase of the splitting cells and the lag phase into the active phase, the fungus divides rapidly and continuing growth into the exponential phase, a phase of multiplication of very large cell numbers. The increasing cell activity greatly influenced by the growing medium such as nutrient content and environmental conditions including temperature and humidity. The following phase was the deceleration phase which the cells begin to be less actively dividing. And then the stationary phase, in this phase the number of cells increased and the number of dead cells relatively balanced but on the 14th day the fungus had an accelerated death phase in which the number of dead or inactive cells was greater than the alive cells still. The results showed that the control media SDA 5.5% to be able to grow Microsporum canis. In SDA media showed the composition media consisting dextrose as a source of carbon and sugar as a source of energy as well as for growth, and protein as energy, and agar as a solidifying media. While the alternative media had composition media that the compotation adjusted into natural resources using some concentration variations. Rice husk can be an alternative replacing SDA media because rice husk powder contains 5.66% carbohydrate as dextrose and 3.78% protein as peptone for growth Microsporum canis. The rice husks powder can be found in various regions of Indonesia Statistical test using normality distribution, homogeneity, and ANOVA showed alternative media of rice husk rice by using variation concentration 4.5%. 5%. and 5.5%, to be able recommended as substitute of SDA media for growth of Microsporum canis (figure 9,10,11). The best optimum concentration 5.5% showed a good result like SDA standard media for the growth of Microsporum canis.

4. Conclusion

Based on experiments that had been done, it can be concluded that rice husk media can be used as alternative media for Microsporum canis growing to replace SDA media. Based on experiments, the best optimum rice husk media concentration for Microsporum canis growing is 5.5%.

5. References

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