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Rice Bran as Opportunity Media for *Candida albicans* Increase

Diah Titik Mutiarawati
Medical Laboratory Technology, Politeknik Kesehatan Kementerian Kesehatan Surabaya, Indonesia;
dihtitikmutiarawati@gmail.com (Corresponding Author)

ABSTRACT

The incidence of invasive fungal infections caused by fungi of the genus Candida is reported to be increasing in various countries. *C. albicans* can be isolated and cultured in liquid media, one of which is PDB *(Potato Dextrose Broth)*, a common medium used for the growth of yeasts and moulds. Bekatul or rice bran is a waste from rice milling, which still contains nutrients such as carbohydrates and protein, which are needed as energy sources for mushroom growth. Colonies were *C. albicans* grown in bran liquid media at mass concentrations of 20 grams, 40 grams, 60 grams, 80 grams and 100 grams with PDB as the *gold standard*. The colonies *C. albicans* growing were then confirmed by planting on PDA media *(Potato Dextrose Agar*), and microscopic testing was performed. The results showed that *C. albicans* grew well in bran liquid medium at pH 5.5-5.0, which was incubated for 1-5 days at a temperature of 25°C-30°C and was characterized by the growth of white colonies on the bottom of the tube, while the number of *C. albicans* that grew on liquid bran media was more than that of PDB media. The conclusion of this research is that the liquid bran media can be used as an alternative medium for PDB for the growth of the fungus *C. albicans*.

Keywords: white rice bran; *Candida albicans*; alternative media; potato dextrose broth

INTRODUCTION

The incidence of invasive fungal infections caused by fungi of the genus Candida is reported to be increasing in various countries. The prevalence of invasive candidiasis is 12.3%, with fairly high mortality and is often found and caused by the fungus *Candida albicans* [(1)]. *Candida albicans* is an opportunistic pathogen and can cause various diseases in humans such as thrush, skin lesions, vulvovaginitis and *gastrointestinal candidiasis* [(2)]. *C. albicans* can be isolated and cultured in liquid media, one of which is PDB *(Potato Dextrose Broth)*, a common medium used for the growth of yeasts and moulds (Himedia, 2015). According to research by Broth [(3)] in their journal, it is said that PDB is used to isolate fungal cells that will be used for molecular examination for identification using PCR. PDB media are instant media made by factories or companies and are already in ready-to-use dosage forms; however, according to store, manufactured media have high prices and can only be found in certain areas. One alternative to get media easily and cheaply is to use unused materials, one of which is organic white rice bran obtained from rice mill waste. Rice is one of the main cereal crops, which is a food product for most of the world's population, especially in Asian countries. The amount of rice harvested worldwide is approximately 600 million tons every year [(4)].

The process of milling rice into rice will produce by-products in the form of waste. The coarsest waste is husk, and the slightly finer one is bran, then the most refined is rice bran (Naim, 2016). Rice bran is easy to find in various regions in Indonesia, has a low price, and is abundant in number, but is considered a less useful material and is usually used as animal feed by the community. The advantages of rice bran, besides being cheap, easy to find in various regions, rice bran also still has nutritional content. Based on research by Manore [(5)], the carbohydrate content in 100 grams of rice bran is 54.6 grams, protein is 12.6 grams, fat is 14.8 grams, and contains vitamin B1 and minerals, namely iron, calcium, phosphorus. Seeing the high carbohydrate and protein content of bran, it is possible that the bran can be used as a source of nutrition for fungal growth. Terms of growth media can grow microorganisms if they contain sufficient nutrients, temperature, appropriate/neutral pH, must be sterile and do not contain inhibitory substances [(6)].

Fungi require nutrients such as water, energy sources, carbon, minerals, growth factors and growth-supporting factors [(7)]. The highest energy for fungal growth are carbohydrates and nitrogen obtained from protein [(8)]. The carbohydrate content in bran is very high, but to be a growth medium, it must have the appropriate proportions for fungal growth. Therefore it is necessary to determine the right amount of bran so that the bran can be used as a fungal growth medium.

Based on this background, it is necessary to conduct further research on organic white rice bran as an alternative liquid medium so that later it can be used as an alternative growth medium for mushrooms.

METHODS

In this study, research was used *experimental*, namely, the research method to determine whether organic white rice bran with the addition of sucrose (sugar) can be used as an alternative liquid medium for the growth of the fungus *Candida albicans* by calculating the pH of the media after growing the fungus, and compared the number of fungal colonies growing on rice bran media with PDB (media *Potato Dextrose Broth*) as the *Gold Standard*.

The population of this study was organic white rice bran obtained from organic rice farmers of the Rojolele type in Madiun Regency and pure culture of *Candida albicans* ATCC 10231 obtained at the Central Health Laboratory of

Surabaya. The sample of this study was organic white rice bran which was used as a liquid medium. In this study, four repetitions of the replication test were carried out from the Federer formula. The total number of samples is 24 tubes.

The independent variable was an alternative medium of organic white rice bran added with sucrose (sugar). The dependent variable in this study was the growth of the fungus *Candida albicans.* The organic white rice bran media was made conventionally; fresh bran was dried in the sun so that it was completely dry and does not clump, then sieved to get fine bran grains. This study used rice bran with mass concentrations of 20 grams, 40 grams, 60 grams, 80 grams, and 100 grams. At each concentration of mass, added with 20 grams of sugar as additional nutrients, then added with distilled water up to 1000 mL. Fungal growth of *Candida albicans was* characterized by the growth of fungus colonies *Candida albicans* on liquid media by forming milky white / cream coloured colonies on the bottom of the tube after incubation at room temperature or around 25-37 C for 1-5 days. Furthermore, *Candida albicans was* transferred to PDA media *(potato dextrose agar)* to confirm the growth of the fungus. The characteristics of colonies that grow on PDA media are cream in colour and have a smooth oval-shaped texture after being incubated at room temperature or around 25-37 C for 1-3 days.

Data collection techniques in this study were by observation, namely by observing the growth of fungal colonies, counting and comparing the growth of colonies *C. albicans* that had been inoculated in alternative liquid media of organic white rice bran and PDB media.

The materials used in this study included the pure culture of the fungus *C. albicans*, organic white rice bran, sucrose (sugar), PDB media, PDA media, antibiotics *chloramphenicol*, *methylene blue*, and aquades. The tools used in this study include a filter, analytical balance, tube, *Petri dish*, ose *loop*, Erlenmeyer, *beaker glass*, funnel, stirring rod, measuring cup, Bunsen, fatty cotton, tripod, and *autoclave.*

Research Procedures

Sterilization of Equipment

Equipment for research in the form of *glassware* and culture media that have been homogeneously sterilized using an autoclave at a temperature of 121 C with a pressure of 1-2 atm for 15 minutes. Sterilization is carried out to avoid contamination of the culture media that will be used in the study.

Preparation of Media

1. Liquid bran: media organic white rice bran liquid media were weighed respectively 20 grams, 40 grams, 60 grams, 80 grams, 100 grams in an Erlenmeyer, then added 20 grams of sucrose and 1000 mL of distilled water into each Erlenmeyer, the media dissolved by heating and should not boil, after dissolving the pH of the media was adjusted to 5.5 and sterilized the media in an *autoclave* at 121 C, for 15 minutes. Next, wait for the media to cool and then add *chloramphenicol* 10 mg offer 100 mL to each medium, then the media is poured into 10 mL sterile tubes.
2. PDB media: PDB media are weighed as much as 24 grams in an Erlenmeyer and dissolved using 1000 mL of distilled water, then the media is dissolved by heating and should not boil, after dissolving the pH of the media is set to 5.5 and sterilizes the media in an *autoclave* at 121 C, for 15 minutes. Next, wait for the media to cool and then add *chloramphenicol* 10 mg offer 100 mL to the media, then the media is poured into a 10 mL tube sterile.
3. PDA media: Fourteen grams of PDA media were weighed in an Erlenmeyer and dissolved using 500 mL of distilled water, then the media was dissolved by heating and should not boil, dissolving the pH of the media was adjusted to 5.5 and sterilized the media in an *autoclave* at 121 C, for 15 minutes. Then wait for the media to cool, then add *chloramphenicol* 10 mg offer 100 mL to the media, then the media is poured in a *sterile Petri dish* 15 - 20 mL.

Data Analysis Techniques

In this study, the presentation of research data is in tabular form. Furthermore, to determine whether the research data is normal or not, the test is carried out *by Kolmogorov-Smirnov*. If the data is normally distributed, the statistical test is continued on the parametric test *one-way ANOVA*, then proceeds to the test *post hoc*, and if it is not normally distributed, it is continued to the non-parametric *Kruskal Wallis Test.*

RESULTS

After conducting research on organic white rice bran liquid media on the growth offungus *Candida albicans* with the gold standard PDB, the following results were obtained:

Table 1. pH of the liquid media of white rice bran and PDB (*potato dextrose broth*)

			pH of the media on day 3			
Replication	PDB	20 g	40 g	60 g	80 g	100 g
1	6	5.5	5.5	5.5	5.5	5.5
2	6	5.5	5.5	5.5	5.5	5.5
3	6	5.5	5.5	5.5	5.5	5.5
4	6	5.5	5.5	5.5	5.5	5.5
			pH of the media on day 4			
Replication	PDB	20 g	40 g	60 g	80 g	100 g
1	6.5	5.0	5.0	5.5	5.0	5.0
2	6.0	5.0	5.0	5.5	5.0	5.0
3	6.5	5.0	5.0	5.5	5.5	5.0
4	6.5	5.0	5.0	5.0	5.0	5.0
			pH of the media on day 5			
Replication	PDB	20 g	40 g	60 g	80 g	100 g
1	6.5	5.3	5.3	5.3	5.3	5.3
2	6.3	5.3	5.3	5.3	5.3	5.3
3	6.5	4.5	5.3	5.3	5.3	5.3
4	6.5	5.3	5.3	5.3	5.3	5.3

After *Candida albicans was* incubated for 3 days in liquid organic white rice bran and PDB (media*Potato Dextrose Broth*), it was continued with confirmation of planting of *Candida albicans* ters. Ebut growing on PDA (media*Potato Dextrose Agar*), after 1 day of incubation on PDA media, the following results were obtained:

Table 2. *Candida albicans* growth on PDA (Potato Dextrose Agar)

Replication	Fungus colonies *Candida albicans*					
	GDP	20 g	40 g	60 g	80 g	100 g
1	193	152	239	304	466	246
2	177	221	155	249	497	249
Average	185	186	197	276	481	247

Data Analysis

From the research data on the growth of the fungus, *C. albicans* on liquid media white rice bran will be tested statistically using statistical tests non-parametric, namely the *Kruskal Wallis Test,* which is used to find out that the data obtained are different.

Data Normality *Kolmogorov-Smirnov* test

Based on the results of the data test, p-value of pH of the media on day 3 was 0.000 (<0.05), pH on day 4 was 0.001 (<0.05), pH on day five that was 0.000 (<0.05) so it could be concluded that the variable data was not normally distributed.

***Kruskal Wallis* test**

Based on the test results, the data showed p-value of pH of the media on day three was 0.000 (<0.05), pH on day 4 was 0.002 (<0.05), pH on day 4. 5 which was 0.001 (<0.05) so it could be concluded that there was a difference in the growth of *Candida albicans* on PDB media with white rice bran liquid media.

DISCUSSION

Liquid Media of Organic White Rice Bran and Growth of Fungus *Candida albicans*

White rice bran liquid media is a medium that contains carbohydrates, proteins, fats, minerals, vitamins, and sucrose as added sugar. Sucrose is a sugar composed of one glucose molecule and one fructose molecule, and sucrose is commonly found in cane sugar or granulated sugar [(9)]. According to Hamad [(10)], granulated sugar as sucrose is easy to find in various regions in Indonesia, and the price is relatively low compared to dextrose. Dextrose is three times more expensive than sucrose, so the use of sucrose as added sugar is more economical than dextrose. In addition to paying attention to the nutritional content, the manufacture of media for fungal growth must also pay attention to pH. This is reinforced by Kuo [(11)] that *C. albicans* has the characteristics of being able to grow fast in acidic conditions (4.5 - 5.5) compared to normal or alkaline pH. Based on the results of the study, it was found that the pH of the liquid bran media for 1-5 days was in an acidic condition and was more acidic than the PDB medium. PDB media on incubation day 3, day 4, and day 5, the pH increased but was still in an acidic condition, while the liquid bran media at concentrations of 20 grams, 40 grams, 60 grams, 80 grams, and 100 grams, pH on The 3rd day was still the same as the initial pH of 5.5 than on the 4th and 5th day, the pH of the media decreased and became more acidic. Based on these results, it can be seen that the growth of fungi in liquid white rice bran media was faster than PDB media. The pH of the liquid bran media changes to acid. It is possible that there is the metabolic activity by the fungus *C. albicans* in the process of breaking down organic matter into an energy source. The difference in pH changes in the liquid media of bran and PDB is due to differences in the nutrient content that composes bran and potato. This is supported by the statement of Amabebe [(12)], that the occurrence of candidiasis due to the presence of *C. albicans* in the vagina can affect pH, changes in pH due to the breakdown of glycogen into lactic acid.

Growth of *Candida albicans* from PDB Media and Bran Liquid Media after being Planted on PDA Media

According to the research results, white rice bran liquid media is more fertile than PDB media. This can be seen from the average number of fungal colonies *C.albicans* that grow on PDA media. In liquid bran media with concentrations of 20 grams, 40 grams, 60 grams, 80 grams, and 100 grams, the average number of colonies was more than PDB media but decreased growth at a concentration of 100 grams, while the highest number of colonies was from liquid media. Bran with bran concentration of 80 grams, the number of colonies, was 481, while from the PDB media, the colonies were 185. Fungal colonies *C. albicans* on bran liquid media were seen more than the growth of the colonies on PDB media. This can be influenced by the presence of nutrients in it, such as carbohydrates and protein in high bran. Based on the results of the examination, carbohydrates were 48.52% and protein 11.97%. Carbohydrates are the main carbon source for the growth of fungi. Carbon compounds are used by fungi to make new cell material, ranging from simple molecules such as simple sugars, organic acids, short-chain and long-chain polymers containing carbon to complex compounds such as carbohydrates, proteins, fats, and acids—nucleic acid. In the process of carbohydrate metabolism, it acts as chemical energy and provides carbon for the assimilation of fungal cell constituents. Proteins are broken down by fungi and used as nitrogen and carbon sources depending on the activity of proteolytic enzymes or proteases [(13)]. Carbohydrates are classified according to the size of their constituent molecules and the groups they contain. Based on the size of the molecule, carbohydrates are divided into two, namely simple sugars and complex carbohydrates, which are called simple sugars are monosaccharide groups. Complex carbohydrates are composed of two or more simple sugars [(14)]. The amount of carbohydrates greatly affects the growth of fungi, but too many

carbohydrates or sugar content can cause a decrease in fungal growth. This has been explained in the journal Rahmat Hidayat that the cause of the decline in fungal growth is because the media contains high glucose levels so that extra yeast cells *C. albicans* experience shrinkage in fungal cells *C. albicans* [(15)].

CONCLUSION

Based on the results of the study, it can be concluded that organic white rice bran liquid media can be used as a growth medium for the fungus *Candida albicans*, which is characterized by the growth of colonies on the media, and its growth is faster than PDB (media*Potato Dextrose Broth*)which is characterized by pH on liquid bran media is more acidic. Organic white rice bran media is more economical because the ingredients are easy to get and cheap. Colonies on organic white rice bran media with mass concentrations of 20 grams, 40 grams, 60 grams, 80 grams, and 100 grams grew more fertile, and the average number of colonies was more than PDB (*Potato Dextrose Broth*) media after confirmation on PDA media *(Potato Dextrose Agar)*

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