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Influence of the Growth of *Candida albicans* on Several Alternative Medium

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***Abstract*—Objectives: The purpose of this study was to determine the effect of some medium on tubers of decoction water on the growth of *C. albicans* and find out the type of medium of tubers boiling water which produced the best growth of *C.albicans* colonies. This study used the experimental method of Completely Randomized Design (CRD) with repetitions of 5 times so that the total number of 25 experimental units was obtained in which pure strains of *C.albicans* which were diluted to 10^{-4} were inoculated on each medium using spread plate technique and incubated at 37ºC for 48 hours. The parameters observed were the number of colonies, shape, size, color and sporulation quality (pseudohifa and blastospores). The results of the analysis showed that alternative media of tubers boiled water had an effect on the growth of *C. albicans* and the medium of taro and cassava were better medium than other alternative medium and the quality of spore growth was comparable to the growth of spores in other medium.**

Keywords: **Candida albicans*, alternative medium, tuber***

I. INTRODUCTION

Research in the field of mycology to study the properties possessed by microorganisms such as fungi, especially fungi that are pathogenic, can be done by breeding through growth media. One of the media commonly used for the isolation and identification of fungi, especially yeast and mold, is the PDA (*Potato Dextrose Agar*) media. PDA is a medium consisting of dextrose, potato extract and agar. Potato extract on PDA is a source of carbohydrates or carbon, dextrose functions as an additional nutrient, and agar as compactor on the media. Each of the three components is needed for the growth and development of microorganisms, especially fungi.

Instant PDA media in ready-to-use form is expensive, hygroscopic, and can only be found in certain places. The high price of instant media which ranges between Rp. 500,000,- up to Rp.1,500,000,- per 500 grams and the abundance of natural resources that can be used as microorganism growth media encourages researchers to find alternative media from materials that are easily available and do not require expensive costs and at the same time can reduce overall costs that must be incurred in research.

Some researchers who have conducted research related to alternative media including Irma[1] have conducted experiments using three flour media namely rice flour, corn flour, and cassava flour. All three flour media showed the best results on the growth rate of fungi. Kwoseh et al [2] who utilize carbohydrate sources from cassava starch as a replacement medium for *Aspergilus niger* and *Fusarium oxyporum* cultures with the result that the two fungi can grow well. Many researchers have used various sources of carbohydrates to be used as an alternative medium for PDA. However, in the manufacturing process, most researchers process the raw materials of carbohydrate source into flour or starch so that other waste is left unused. Meanwhile, if it refers to the procedure of making the PDA media itself, carbohydrate substances are obtained through the boiling process so that carbohydrate substances will come out of the cell and bind to water molecules.

Different from previous studies where most researchers used flour products and starch extract as the source of carbohydrates, this time researchers were interested in conducting further research on the use of other carbohydrates sources in the development of alternative media to replace PDA media using boiled water from cassava, sweet potato, taro, and uwi. Cassava is a food source of carbohydrates with a higher carbohydrate content of 34,70 grams per 100 grams cassava, sweet potato contains 27,9 g, taro contains 23,7 g, and uwi contains 19,8 g while potatoes only contain 19,10 g [3].

Utilization of food sources carbohydrates is expected to be able to help in the field of research, especially in the enforcement of diagnosis in candidiasis. Generally, to help support the diagnosis of a disease caused by yeast or mold, Candida albicans is optimally grown on the media of Potato Dextrose Agar (PDA).

Therefore, in this research, *Candida albicans* can be used as a research object of alternative media for PDA replacement. The comparable nutritional content between PDAs and alternative tubers boiled water media is estimated to create optimum conditions for the growth of *Candida albicans*. Therefore, the purpose of this research is to find out whether the tubers bolied water media can affect the growth of *C.albicans* and the types of tubers boiled water media that produce the best growth of *C.albicans* colonies.

II. MATERIAL AND METHOD

A. Procedure

1) *Material Preparation*

The tools used in this research are Autoclave, Basin, Stirring Rod, BSC, Petri Dish Ø 6 cm, Cover glass, Dry Heat Sterilizer, Erlenmeyer, Measuring Cup, Hot plate, Watch Glass, Filter Cloth, Cotton wool, Lighters, Microscopes Binoculars, Analytical balance, Object glass, Straight Ose, Pan, universal pH, Dropper Pipes,

Knives, Spatulas, Spirtus, Reaction Tubes, Cutting Board, and Water bath. The materials used in this research are Bacto Agar, sterile distilled water, Alcohol 70%, Tartrate Acid 1%, Chloramphenicol, Dextrose, Gram Stain, physiological NaCl, PDA, Cassava, pure strain of Candida albicans, Taro, Sweet Potatoes, and Uwi.

2) *PDA Media Making*

3,9 grams of PDA media was dissolved in 100 mL distilled water by heating it on hot plate until it dissolved which was marked by the media becoming clear. The pH of the media was checked using universal pH and 1% tartaric acid was added until pH 5-6 was shown. Media that had been covered with a plug was sterilized using an autoclave with a temperature of 121°C for 15 minutes. Wait until the temperature was lukewarm and then the media was poured into a sterile petri dish.

3) Alternative Media Making

The tubers that had been sorted and peeled were diced and weighed as much as 20 grams. 100 mL of distilled water was added and heated until the tubers were cooked. The boiled water was filtered and distilled water was added up to 100 mL. 2 grams of dextrose and 1,5 grams of jelly (agar-agar) were added. It was heated on a hot plate until it dissolved. The pH was checked and 1% tartaric acid was added until pH 5-6 was shown. Media that had been covered with a plug was sterilized using an autoclave with a temperature of 121°C for 15 minutes. Wait until the temperature was lukewarm and then the media was poured into a sterile petri dish.

4) Quality Control Media

Two cups from each type of media were incubated at 37oC for 24 hours. If the amount of contaminants is more than 10%, then it is concluded that the quality of the media is not suitable for use and the batch must be discarded. (Cowan and Steel's, 1993).

5) *C. albicans* Breeding

C.albicans mushroom strain that had been diluted to 10-4 dilution were inoculated in each medium by spread plate technique using sterile L stems. The media was incubated at 37°C for 48 hours and the colony growth was calculated every 24 hours and the color and diameter of the colony was observed. After 48 hours, the growing colonies were observed microscopically using gram staining techniques and the quality of the blastosopra and its pseudohifa.

B. Data Analysis[4]

The data obtained were then analyzed statistically using the one way ANOVA test and were also analyzed using Duncan's Multiple Range Test (DMRT) at an error rate of 5% using the IBM SPSS Statistic 24.0 program.

III. RESULTS

The test results show that *C.albicans* can grow well in all treatments. Furthermore, the data obtained were processed statistically using the One Way ANOVA test to determine the effect of alternative media on the growth of *C.albicans*. Based on the results of the one way ANOVA analysis, it showed a significant value ($p<0,05$) for the type of media tested, it showed that there were significant differences form the treatments given in which tubers boiled water media could influence the growth of *C.albicans*. The results of the ANOVA test were followed by the Duncan test to determine differences in the growth of *C.albicans* colonies in each treatment. The detailed result is elaborated in the following Figure 1.

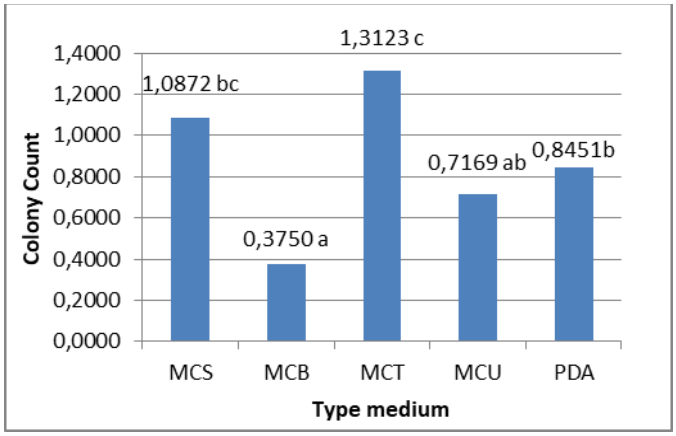

Figure 1: Histogram DMRT test results on the average growth of colonies in various treatments

Note : a, b, c: cluster symbol on the DMRT test results from low to high. Numbers followed by the same letter do not differ at a 5% error rate
MCS: *C.albicans* on Cassava Medium.
MCB: *C.albicans* on Tuber Medium.
MCT: *C.albicans* on Taro Medium.
MCU: *C.albicans* on Uwi Medium.
PDA: *C.albicans* on *Potato Dextrose Agar*

Further test results showed a different colony growth rate for each treatment. It is known that the type of treatment that showed the highest colony growth was the MCT (medium of taro boiled water) treatment reaching 1,3123. Statistically, through the DMRT test with a significance level of 5% shows that the MCT treatment was significantly different from other treatments except MCS (medium of cassava boiled water) with a significance value of 0,210. However, the MCS treatment showed also no significant difference on PDAs and MCU (media of uwi boiled water) with a significance value of 0,056, and MCU showed also no significant difference with MCB (medium of sweet potato boiled water) with a significance

value of 0,063. These results indicate that the medium of taro and cassava boiled water are better media in growing *C.albicans* that other alternative media.

Macroscopically, the *C.albicans* colonies that grow in each treatment showed a colony growth with a round shape, soft texture, creamy color, flat edges and with the diameter of 2-3 mm. This is in accordance with the explanation of Jawetz, et al (2013) who mentioned that in agar media, in 24 hours, at 37oC or at room temperature, Candida species produce creamy soft colonies with the smell of yeast. The growth can be observed by the increase in colony diameter in the growth media. At the age of mushroom in 24 hours, the colony diameter is still small, that is around 1-2 mm. Then after 48 hours, the colony diameter gets bigger, that is around 2-3 mm. This is consistent with the statement of Gandjar (2006) which states that one of the growth parameter is the increase in cell volume. In general, colonies originate from a cell which is not initially seen to be visible, that is from spores or conidia fungal to mycelium or colonies. The additional of the colony is irreversible meaning it cannot return to its original volume. The macroscopic shape of *C.albicans* colony can be seen in the figure 2.

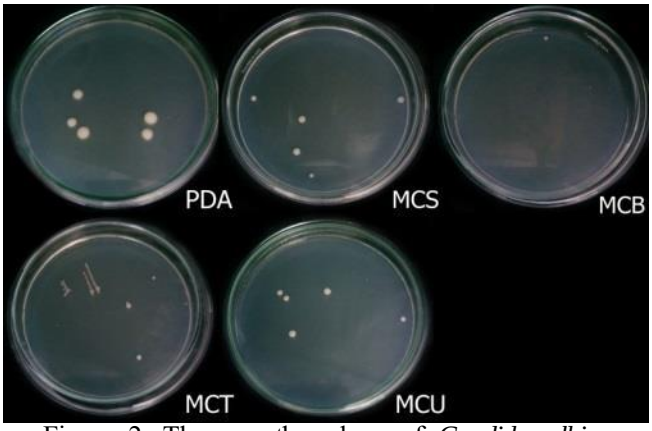

Figure 2. The growth colony of *Candida albicans* on several alternative media

Note : MCS: *C.albicans* on Cassava Medium.
MCB: *C.albicans* on Tuber Medium.
MCT: *C.albicans* on Taro Medium.
MCU: *C.albicans* on Uwi Medium.
PDA: *C.albicans* on *Potato Dextrose Agar*

Microscopically, a yeast cell with an oval round shape, with sprout and purple in color was found. This is consistent with the characteristics of *C.albicans* described by Jawetz et al [5] who states that Candida species grow in an oval shape, small, thin-walled, with sprout, gram-positive, in the size of 3-6 µm. The Candida shape can be seen microscopically in Figure 3 as follows.

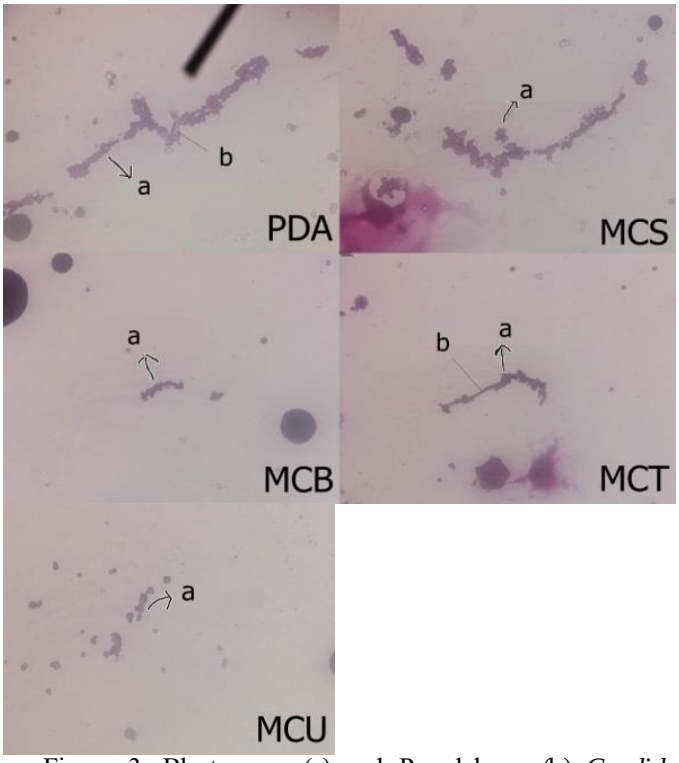

Figure 3. Blastospora (a) and Pseudohypa (b) *Candida albicans* on several alternative media

Note : MCS: *C.albicans* on Cassava Medium.
MCB: *C.albicans* on Tuber Medium.
MCT: *C.albicans* on Taro Medium.
MCU: *C.albicans* on Uwi Medium.
PDA: *C.albicans* on *Potato Dextrose Agar*

IV. DISCUSSION

Factors that can affect the growth of colonies in each treatment are nutrient content, tubers maturity level, and fiber content in tubers [6]. Each 100 grams of cassava contains 34,7 grams of carbohydrates, sweet potato contains 27,9 grams of carbohydrates, taro contains 23,7 grams of carbohydrates, and uwi contains 19,8 grams of carbohydrates, while potato contains only 19,1 grams of carbohydrates [3]. The amount of growth of *C.albicans* colonies in taro boiled water alternative media showed the highest amount of growth, this also shows that the composition of nutrients, especially carbohydrates in the media is in accordance with *C.albicans* nutritional needs. Amir et al [7]explained that carbohydrates are sugar molecules or a combination of sugar molecules that have many types. Based on the constituent sugars, carbohydrates are classified into monosaccharide, disaccharides, oligosaccharides and polysaccharides. The types of carbohydrates in taro and cassava are starches classified as polysaccharides and are generally reserve materials in the body of plants.

Koswara [8]explains that the starch content in taro tubers is also high ranging between 75-80%. Amylose and amylopectin are components of taro starch. Amylose content in taro starch is 20-25%[8] and amylopectin is 78,56%[9]. Whereas in the cassava, the content of amylose is 18% and amylopectin is

60,15% [10]. Amylose is a fraction that is soluble in water, has a straight structure with α-(1,4)-glycosidic bonds with a length between 250-2000 units of D-glucose[11]. While amylopectin is a fraction that is more difficult to dissolve in water, has the same structure as amylose in large quantities but has a branching with a α-(1,6)-glycosidic at its branching with the number of 20-25 units of D-glucose per branch which cause its bond to be stronger and the molecular structure is more stable so it is difficult to dissolve in water [12]. The comparison between amylose and amylopectin will also affect the solubility and gelatinization of strach. Iwuoha dan Kalu [13] describe gelatinization as swelling of the granules so that they cannot return to their original shape. With gelatinization, there is also a change in the viscosity of starch. The longer the heating will cause the higher viscosity. When the starch solution reaches gelatinization temperature, the starch granules will break. This causes water to enter and hydrolyze starch into a simpler form, which is glucose. The gelatinization temperature of taro tuber starch was at 70°C in 194 seconds, while cassava starch was at 68-78°C in 326 seconds . The difference in temperature and gelatinization time in the taro tuber extraction process which is faster than cassava tubers is estimated to contain starch hydrolysis result in the form of glucose which is more than in cassava boiled water so that *C.albicans* is able to grow better than in cassava boiled water media. The high carbon content in taro is also what causes *C.albicans* to grow beyond the growth in PDA media as well as cassava.

Each 100 grams of taro contains 0,2 grams of fat, cassava contains 0,3 grams of fat, potato contains 0,1 gram of fat, uwi contains 0,2 grams of fat and sweet potato contains 0,7 grams of fat [3]The amount of *C.albicans* colony growth in the alternative medium of sweet potato boiled water showed the lowest amount of growth even though sweet potatoes contained high carbohydrate levels, this could be due to the fat content in sweet potatoes. The presence of fat in sweet potatoes boiled water media can affect cell surface tension and cell permeability membranes, whereas *C.albicans* does not have enzymes that can hydrolyze fat so that nutrients are difficult to be absorbed into the cells[7].

The growth and development of *C.albicans* is also influenced by environmental conditions such as humidity, temperature, and the pH of the media. During the research, the incubator humidity was around 68-74% with the temperature range of 35,9 – 36,7°C, and the pH of the media was 5. According to Siregar (2004) *Candida albicans* grew optimally at temperatures between 28-37°C with degree of acidity ranging from 4,5-6,5.

V. CONCLUSION

Based on the results of the research and discussion, it can be concluded that the alternative media of tubers boiled water influences the growth of Candida albicans colonies and alternative media of taro and cassava boiled water has better growth compared to other alternative media. While the quality of the spores produced is as good as the Candida albicans spores produced by other media.

This research is expected to be used as a reference in making alternative substitutes for PDA and further research is conducted on the stability of this alternative media to find out how long this media can still be used to grow fungi of the same quality, the growth of other fungi on this alternative media and the further test on the water content of tubers boiled water.

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REFERENCES

[1] Irma, "Optimasi Media Pertumbuhan Aspergillus niger Dengan Menggunakan Tepung Singkong," *Development*, vol. 134, no. 4, 2015.

[2] C. K. Kwoseh, M. Asomani-Darko, and K. Adubofour, "Cassava starch-agar blend as alternative gelling agent for mycological culture media," *Bots. J. Agric. Appl. Sci*, vol. 8, no. 1, 2012.

[3] M. Astawan, *Panduan Karbohidrat Terlengkap*. Jakarta: Dian Rakyat, 2009.

[4] Khusnul, R. Suhartati, D. Virgianti, M. Fathurohman, and A. Pratita, " Effect of Karuk Leaves (Piper Sarmentosum Roxb) and White Galangal Rhizome (Alpinia Galanga L) Ethanol Extract on the Growth of Microsporum Gypseum and Candida Albicans in Vitro ," *J. Phys. Conf. Ser.*, vol. 1179, p. 012168, 2019.

[5] Jawetz, Melnick, and Adelberg's, *Medical Microbiology, 26th Ed.* United States: The McGraw-Hill Companies, Inc, 2013.

[6] N. Aini and T. Rahayu, "Alternatif Media for Fungal Growth Using a Different Source of Carbohidrats Nurul," *Semin. Nas. XII Pendidik. Biol. FKIO*, pp. 861–866, 2015.

[7] Amir, N. I. Sari, Darmawati, and S. S. Dewi, "Tepung Talas sebagai Media Alternatif Pertumbuhan Candida albicans dan Aspergillus sp.," *Pros. Semin. Nas. Mhs. Unimus*, vol. 1, pp. 78–85, 2018.

[8] Koswara, *Teknologi Pengolahan Umbi-umbian*. Jakarta: USAID, 2013.

[9] S. ; T. K. P. Hartati, "Analisis Kadar Pati dan Serat Kasar Tepungbeberapa Kultivar Talas (Colocasia esculenta L. Schott)," *J. Natur Indones.*, vol. 6, pp. 29–33, 2003.

[10] Nisah and Khairun, "Study Pengaruh Kandungan Amilosa dan Amilopektin Umbi-umbian Terhadap Karakteristik Fisik Plastik Biodegradable dengan Plastizicer Gliserol.," *J. Biot.*, vol. 5, pp. 106–113, 2017.

[11] O. Fennema, *Principle of Food Science. Part I Food Chemistry*. New York: Marcel Dekker inc, 1976.

[12] F. Winarno, *Kimia Pangan dan Gizi*. Jakarta: PT Gramedia Pustaka Utama, 1992.

[13] K. F. Iwuoha CI, "Calsium oxalate and physico-chemical properties of cocoyam (Colocasia esculenta and Xanthosoma sagittifolium) tuber flours as affected by processing," *Food Chem.*, vol. 54, pp. 61–66, 1995.