

CORRELATION BETWEEN *CANDIDA ALBICANS* COLONIZATION GROWTH AND THE ADDITION OF 5%, 10% AND 40% DEXTROSE LEVEL ON SABORAUD DEXTROSE AGAR

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ABSTRACT

Introduction : *Candida albicans* is normal flora that can be found on skin, oral mucosa and digestive tract. This organism are commensal or non pathogenic, but when there is other predisposition factor, this commensal tendency may become pathogenic to the human body. In *Diabetes Mellitus* patients with high blood sugar level can affect *Candida Albicans* infection events.

Aim: The purpose of this research is to prove whether there is a correlation between *Candida Albicans* colonization growth and the addition of 5%, 10% and 40% *Dextrose* level on *Saboraud Dextrose Agar* (SDA).

Method: This research is a true experimental research with post test only group design observed in 11 days. Samples are from 15 strains of *Candida Albicans* cultured in *Saboraud Dextrose Agar* without the addition of *Dextrose*, with the addition of *Dextrose* 5%, 10% and 40% in eleven days and the development were noted. The growth of *Candida albicans* is observed from the diameter growth.

Result: in the culture of *Candida albicans* in 11 days. Data analysis using *Kruskal Wallis* with data signification ($\alpha = 0,05$) obtained P 0,000 ($P < 0,05$), which means there is a significant correlation between *Candida Albicans* colonization growth and the addition of 5%, 10% and 40% *Dextrose* level on *Saboraud Dextrose Agar*.

Conclusion: the best addition in this research is the addition of 5% *Dextrose* on *Saboraud Dextrose Agar*.

Keywords: *Candida albicans*, Addition, *Dextrose*, *Saboraud Dextrose Agar* (SDA).

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INTRODUCTION

Candidiasis is an infection caused by the fungus of the genus *Candida*.^(1,2) Candidiasis is included in one of the superficial dermatomycosis that often occurs in humans.⁽³⁾ Some *Candida* species which is often found in humans and animals is *Candida albicans*.⁽⁴⁾ This organism can infect the skin, nails, mucous membranes, gastrointestinal tract, and can even cause systemic diseases.⁽⁵⁾

Candida albicans or other species of *Candida* are normal flora that are present in the skin, oral cavity and digestive tract. Under normal circumstances, *C. albicans* is found in 80% of healthy people.^(1,5) This organism belongs to the commensal organism which means it is present in the body under normal conditions and is non-pathogenic.^(6,7) And it has opportunistic properties, so that if there are predisposing factors, which can be derived from endogenous or exogenous factors, may cause changes in the nature of *C. albicans* from commensal to pathogenic.^(8,9)

Exogenous factors originate from environmental conditions that support *C. albicans* characteristic e.g. climate. Endogenous factors originating from the host's body, such as endocrine disorders, namely diabetes mellitus (DM) can be related to the growth of *C. albicans*.^(2,10) The growth of *C. albicans* in DM patients is associated with high blood glucose levels.⁽¹¹⁾ A 2011 study by Han et al., found that carbohydrates are needed both for cellular growth and for the transition of *C. Albicans* to fungal forms.⁽¹²⁾ *Candida* species are considered important pathogens because of their flexibility and ability to survive in various anatomical sites.⁽¹³⁾

A 1992 study by Aly et al showed that in patients with unregulated diabetes mellitus had a significant relationship between blood glucose levels and growth of *C. albicans*. These levels are in accordance with the expert's opinion that high blood glucose levels that are uncontrolled affect the incidence of *C. albicans* infection.⁽¹⁴⁾

Research from the United States by Vargas et al., found evidence in experiments of rats on a high-glucose diet experiencing increased colonization and invasion by *Candida* in the intestine.⁽¹⁵⁾ An American study by Weig et al. Also evaluated the effects of a high-sugar diet to the number of *Candida* cells in the human intestine and found an increase in the number of *Candida* cells in the stool after a high-sugar diet. This study stated that the people tested here had normal immunity.⁽¹⁶⁾

In the process of fungal culture *C. albicans* takes up to 72 hours of incubation time at a temperature of 25 ° - 30 ° C. *C. albicans* mushroom culture uses SDAB (Sabouraud Dextrose Broth) or SDA (Sabaroud Dextrose Agar) media, the latter is usually used more often. The addition of dextrose in this study is intended to increase fertility levels in SDA (Sabaroud Dextrose Agar) media which will help the growth of fungal colonies *Calbicans* to be more fertile.

METHODS

This study used true experimental design with post test only control group design. This study used *C albicans*, each chosen randomly (R). There are two groups: treatment and control. The study population was a *Candida* sp colony that had been cultured at the Laboratory of the

Faculty of Medicine, Widya Mandala Catholic University, Surabaya, Pakuwon City campus. The sample from this study was a specific colony of *C. albicans* which had been germ tube tested from the results of *Candida sp* culture. The sampling technique in this study was Simple Random Sampling from the colonies of *Candida sp*. The inclusion criteria of this study were specific colonies of *C. albicans* which grew on Sabouraud Dextrose agar media. Whereas the exclusion criteria are colonies that are not specific to *C. albicans*. The material for the study was taken from pure *C. albicans* strains. *C. albicans* strains was cultured on Sabouraud Dextrose agar without the addition of dextrose (which has 4% dextrose in it) and treated to 5% dextrose, 10% dextrose, and 40% dextrose. Sabouraud Dextrose agar without additional dextrose treatment was the control in this study to be compared with so that those given 5%, 10%, and 40% additional dextrose levels. Examination of culture is done by macroscopic observation for the presence of fungal growth. *Candida sp. albicans* and *non albicans* will be identified using the germ tube

test. The growth of *C. albicans* was observed, noting the difference from the results of the culture in Sabouraud Dextrose agar without the addition of dextrose and with the addition of different dextrose. Sabouraud Dextrose agar was tested for Specificity and Sensitivity. Data was analyzed using chi-square.

RESULT

In table 1 shows a comparison between the growth of *C. albicans* on Sabouraud Dextrose (SDA) agar media in the treatment and control groups, from the first day to the eleventh day in millimeters. From the table above, it can be seen that the *C. albicans* growth was the best in the 5% dextrose treated group, followed by the 10% dextrose treated, followed by control, and last the 40% dextrose treated group. Better growth seen through large diameter growth starting from the first day to the last day. Up to day eleven, the growth of *C. albicans* in the 5% SDA media remained larger than the SDA Control and with the addition of 10% and 40% dextrose.

Table 1. Growth of *Candida Albicans* Until Day 11

Strain <i>Candida albicans</i>	Pertumbuhan <i>Candida albicans</i> (mm)																			
	Hari 1				Hari 3				Hari 7				Hari 9				Hari 11			
	K	5%	10%	40%	K	5%	10%	40%	K	5%	10%	40%	K	5%	10%	40%	K	5%	10%	40%
A	3	3	3	3	7	8	7	5	11	13	12	8	13	17	15	10	15	19	17	12
B	4	4	4	2	7	8	8	5	11	13	12	7	13	16	15	9	16	19	17	10
C	3	4	3	3	6	7	7	5	10	12	11	8	12	14	13	10	14	16	15	11
D	4	4	4	3	7	7	7	5	11	13	12	8	13	16	15	9	15	19	17	10
E	3	4	4	3	6	7	7	5	11	13	12	9	13	16	15	11	15	19	18	12
F	3	4	3	3	6	7	7	5	10	13	12	8	13	16	14	10	15	18	17	11
G	3	4	3	3	7	7	7	6	11	13	12	10	13	16	15	12	17	20	19	14
H	3	5	4	3	6	8	7	5	11	14	12	8	13	17	15	10	16	20	18	11
I	3	4	3	3	7	8	7	5	11	14	13	9	13	16	15	11	15	19	17	12
J	3	4	3	3	6	7	7	5	11	13	13	10	13	16	15	12	16	18	17	13
K	3	4	4	3	6	8	7	5	11	14	12	10	13	17	15	12	16	20	18	13
L	4	5	5	4	7	9	8	6	12	15	14	10	14	17	15	13	18	20	19	15
Ket.	5%>10%>K>40%				5%>10%>K>40%				5%>10%>K>40%				5%>10%>K>40%				5%>10%>K>40%			

Shapiro Wilk test was used to test for normality because the number of samples was no more than 50

Table 2. Normality test

Tests of Normality

Shapiro-Wilk		
	Statistic	Sig
Day 1	,767	,000
Day 3	,888	,000
Day 7	,959	,040
Day 9	,956	,032
Day 11	,935	,003

Table 2 shows that on days 1, 3, 7, 9, and 11 of the growth of 15 strains of *C. albicans* did not have a significance value of $p > 0.05$, so it can be said that the growth variable *C. albicans* was not normally distributed so hypothesis testing was carried out using non parametric test, namely the Kruskal Wallis test.

Homogeneity test was carried out using Levene. The significance value of the Levene homogeneity test is $p > 0.05$. As shown in table 3 it can be concluded

Table 3. Homogeneity test
Test of Homogeneity of Variances

Levene test		
	Statistic	Sig
Day 1	4,195	,010
Day 3	2,273	,090
Day 7	4,378	,008
Day 9	5,204	,003
Day 11	2,154	,104

that there were those that did not fulfill the homogeneity assumption of Kruskal Wallis test. Chi-square test results shown in table 4, it shows that there is a

significance value of $p < 0.001$ suggest that there was a significant relationship between the growth of *C. albicans* colonies with the addition of Dextrose levels of 5%, 10%, and 40% on Sabouraud Dextrose agar.

Table 4. Different Test

	Day 1	Day 3	Day 7	Day 9	Day 11
Chi-square	19,57	36,28	41,01	42,16	38,12
Asymp. Sig.	,000	,000	,000	,000	,000

DISCUSSION

In the study it was shown that there was a significant relationship between the growth of *C. albicans* colonies with the addition of Dextrose 5%, 10%, and 40% on Sabouraud Dextrose agar. The result suggested that there were a significant correlation between *C. albicans* growth and concentration of Dextrose treatment. Data from this study supported the research reported by Samarayanake et al. that saliva used as a growth medium requires glucose for the growth of *Candida albicans*.¹⁹

The growth of *C. albicans* was also influenced by the duration of exposure to Dextrose. The duration of exposure to glucose for eleven days increased compared to the previous day. This data confirmed that candidiasis is more often found when the availability of glucose with a high enough level in a long time, such as in patients with Diabetes Mellitus.¹⁴

The growth of *C. albicans* in SDA 5% was larger in diameter starting from the first day. On the eleventh day, the growth of *C. albicans* in the 5% SDA media remained larger than SDA without dextrose addition.

Table 5. Growth of *Candida albicans* between control and addition of 5% Dextrose.

Strain <i>Candida albicans</i>	Growth of <i>Candida albicans</i> (mm)									
	Day 1		Day 3		Day 7		Day 9		Day 11	
	K	5%	K	5%	K	5%	K	5%	K	5%
A	3	3	7	8	11	13	13	17	15	19
B	4	4	7	8	11	13	13	16	16	19
C	3	4	6	7	10	12	12	14	14	16
D	4	4	7	7	11	13	13	16	15	19
E	3	4	6	7	11	13	13	16	15	19
F	3	4	6	7	10	13	13	16	15	18
G	3	4	7	7	11	13	13	16	17	20
H	3	5	6	8	11	14	13	17	16	20
I	3	4	7	8	11	14	13	16	15	19
J	3	4	6	7	11	13	13	16	16	18
K	3	4	6	8	11	14	13	17	16	20
L	4	5	7	9	12	15	14	17	18	20
Ket.	K < 5%		K < 5%		K < 5%		K < 5%		K < 5%	

The growth of *C. albicans* in SDA 10% was larger in diameter starting from the first day. On the eleventh day the

growth of *C. albicans* in the 10% SDA media remained larger than SDA without dextrose addition.

Table 6. Growth of *Candida albicans* between control and addition of Dextrose 10%.

Strain <i>Candida albicans</i>	Growth of <i>Candida albicans</i> (mm)									
	Day 1		Day 3		Day 7		Day 9		Day 11	
	K	10%	K	10%	K	10%	K	10%	K	10%
A	3	3	7	7	11	12	13	15	15	17
B	4	4	7	8	11	12	13	15	16	17
C	3	3	6	7	10	11	12	13	14	15
D	4	4	7	7	11	12	13	15	15	17
E	3	4	6	7	11	12	13	15	15	18
F	3	3	6	7	10	12	13	14	15	17
G	3	3	7	7	11	12	13	15	17	19
H	3	4	6	7	11	12	13	15	16	18
I	3	3	7	7	11	13	13	15	15	17
J	3	3	6	7	11	13	13	15	16	17
K	3	4	6	7	11	12	13	15	16	18
L	4	5	7	8	12	14	14	15	18	19
Ket.	K < 10%		K < 10%		K < 10%		K < 10%		K < 10%	

The growth of *C. albicans* appeared to be inhibited on day 1-3 as the diameter of the specimen treated to 40% dextrose was smaller than the control. This might be caused by plasmolysis. When

cells are placed in a solution that has a higher concentration of solute or

hypertonic than the cell, then the cell will experience plasmolysis. Plasmolysis is the event of shrinking the cytoplasm, loss of

water and detachment of the plasma membrane from the plant cell wall if the cell is inserted into a hypertonic solution. Plasmolysis is the effect of an osmosis event. The greater the proportion of

Dextrose added, the greater the incidence of plasmolysis that occurs. Plasmolysis can occur in walled cells ranging from bacteria, fungi and plant cells.

Table 7. Growth of *Candida albicans* between control and addition of Dextrose 40%.

Strain <i>Candida albicans</i>	Growth of <i>Candida albicans</i> (mm)									
	Day 1		Day 3		Day 7		Day 9		Day 11	
	K	40%	K	40%	K	40%	K	40%	K	40%
A	3	3	7	5	11	8	13	10	15	12
B	4	2	7	5	11	7	13	9	16	10
C	3	3	6	5	10	8	12	10	14	11
D	4	3	7	5	11	8	13	9	15	10
E	3	3	6	5	11	9	13	11	15	12
F	3	3	6	5	10	8	13	10	15	11
G	3	3	7	6	11	10	13	12	17	14
H	3	3	6	5	11	8	13	10	16	11
I	3	3	7	5	11	9	13	11	15	12
J	3	3	6	5	11	10	13	12	16	13
K	3	3	6	5	11	10	13	12	16	13
L	4	4	7	6	12	10	14	13	18	15
M	3	3	7	6	11	9	14	12	17	15
N	3	3	7	6	12	9	13	12	17	15
O	3	3	7	5	11	9	14	11	18	12
Ket.	K > 40%		K > 40%		K > 40%		K > 40%		K > 40%	

CONCLUSION

This study confirmed that there is a relationship between the growth of *C. albicans* and the addition of Dextrose addition of 5%, 10% to *C. albican* cultures led to more fertile growth compared to standard Sabouraud Dextrose agar media. Sabouraud Dextrose media with the addition of 5% dextrose led to the most optimal growth, therefore should be used as the preferred mushroom culture medium, which can help speed up the diagnostic time in patients suspected of having *C.albicans*.

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