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Differences in the growth of *Candida albicans* fungi on alternative media of soybean (*Glycine max* (L.) Merrill) and Sabouraud Dextrose Agar (SDA)

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Article

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Abstract

Candida albicans is an opportunistic pathogenic fungus that can cause candidiasis. However, culture media commonly used for its growth, such as Sabouraud Dextrose Agar (SDA), are relatively expensive and not always readily available. Soybeans (*Glycine max* (L.) Merrill) contain various nutrients, including carbohydrates, proteins, fats, vitamins, and minerals, which may support fungal growth and serve as an alternative culture medium. This study aimed to determine the difference in the growth of *Candida albicans* on an alternative soybean medium and SDA. A quantitative experimental approach with a Post-Test Only Control Group Design was employed. The study used *Candida albi*cans ATCC 14053 isolates, which were inoculated onto both the soybean alternative medium and SDA and incubated for 24 hours. Fungal growth was evaluated by counting the number of colonies formed on each medium. The results showed that the mean number of *Candida albicans* colonies on the soybean medium was 98 colonies, whereas the mean number on SDA was 31 colonies. Statistical analysis using the Independent Samples t-test revealed a significant difference in fungal growth between the two media ($p = 0.002$; $\alpha = 0.05$). These findings indicate that the soybean alternative medium supports the growth of *Candida albicans* significantly better than SDA and has potential as a cost-effective and environmentally friendly alternative culture medium for fungal cultivation.

INTRODUCTION

Candida albicans is a dimorphic microscopic fungus that normally lives as a commensal in the human gastrointestinal tract, oral cavity, and reproductive tract. However, under immunocompromised conditions or when the body's microflora balance is disturbed, this fungus can transform into an opportunistic pathogen, causing candidiasis infections, ranging from superficial infections of the skin and mucosa to life-threatening systemic infections [1]. Although generally harmless in healthy individuals, changes in the body's microenvironment or a decline in the immune system (immunocompromise) can trigger the pathogenic transition of this fungus from a yeast form to an invasive hypha [2]. This transformation triggers candidiasis, ranging from superficial infections such as oral thrush and vulvovaginitis to severe systemic infections associated with high morbidity in hospital settings [3]. The increasing prevalence of infections caused by *Candida albicans* demands rapid and accurate laboratory diagnosis, one of which is through the culture method [4].

Indonesia has a humid tropical climate, creating ideal conditions for the growth of various microorganisms, including fungi. One significant type of fungus in human infections is *Candida albicans*. This fungus can cause an infection known as candidiasis [5]. According to the World Health Organization (WHO), candidiasis is a common fungal infection, with a prevalence of 10–15% among women worldwide each year. The incidence of candidiasis remains high annually, particularly in Indonesia, which has a tropical climate characterized by high temperatures and humidity. Between 2011 and 2013, there were 114 patients with skin infections and 23 with nail infections; the majority were

women: 54.3% in 2011, 80% in 2012, and 56.6% in 2013 [6]. The prevalence of candidiasis in Indonesia, particularly among women, is 80–90% [7].

If left untreated, *Candida albicans* fungal infections can progress into chronic infections and worsen over time. Given the environmental conditions that support this fungus's growth, it is important to understand the risk factors and implement preventive measures to reduce the incidence of infection [8]. Laboratory diagnosis of candidiasis can be performed by culturing patient specimens. In the culture system for candidiasis, *Candida albicans* is isolated from patient samples on a medium. One effective synthetic medium for the growth of *Candida albicans* is SDA [5].

In microbiology laboratories, Sabouraud Dextrose Agar (SDA) has long been the gold standard medium for fungal isolation and cultivation. SDA medium has an optimal composition that supports fungal growth, primarily due to its peptone content as a nitrogen source and high dextrose concentration as a carbon source [9]. However, reliance on commercial media such as SDA faces several practical challenges, including relatively high cost, often limited availability in laboratories in remote healthcare facilities, and limited shelf life [10,11].

Aini [12] explained that Sabouraud Dextrose Agar (SDA) is available as a commercially prepared medium produced by specialized manufacturers and is widely used for fungal cultivation. However, its availability may be limited in certain educational and regional laboratories because it depends on procurement through laboratory reagent suppliers and is not always readily accessible in all locations. In addition, the use of commercially manufactured media can increase laboratory operational costs, particularly for routine microbiological examinations and research activities conducted in resource-limited settings. These limitations have encouraged researchers to explore alternative culture media derived from natural materials that are more accessible and locally available. Natural ingredients containing carbohydrates, proteins, and lipids have been reported to support microbial growth and therefore have potential as alternative media. Soybean (*Glycine max* (L.) Merrill), which is rich in essential nutrients, is one such material that may be developed as an alternative medium for the cultivation of *Candida albicans*. This problem has prompted researchers to explore the use of local natural products that are economical, easy to obtain, and rich in nutrients as alternative media formulations for mushroom cultivation [13,14].

Research conducted by Badrud Tamam [15] demonstrated that soybean-based medium (*Glycine max* (L.) Merrill) can be used as an alternative medium for culturing *Candida albicans*. Similarly, Rahman et al. [16] reported that soybean meal supported the growth of *Candida albicans*, particularly at concentrations of 5% and 7%. These findings indicate that *Candida albicans* is capable of utilizing the complex nutritional components of soybeans to support its growth and development. Although the potential of soybean-based media for cultivating *Candida albicans* has been previously reported, studies evaluating specific soybean varieties as alternative culture media remain limited. In particular, the growth-supporting capability of the Grobogan soybean variety has not been widely investigated or directly compared with the standard Sabouraud Dextrose Agar (SDA) medium. Therefore, this study aimed to evaluate the growth of *Candida albicans* on an alternative medium prepared from Grobogan soybeans and to compare its performance with SDA as the reference medium.

Soybeans (*Glycine max* (L.) Merrill) are one of the most widely consumed plant-based protein sources in Indonesia and have considerable potential as an alternative medium for fungal cultivation, particularly as a substitute for SDA [17]. Soybeans contain essential nutrients, including carbohydrates (35%), proteins, lipids (18%), and minerals (5%), which may support the growth of microorganisms [18]. For *Candida albicans*, carbohydrates serve as a primary carbon source for cellular metabolism and energy production, while proteins provide nitrogen required for the synthesis of cellular components and fungal growth. In conventional SDA medium, dextrose functions as the main carbon source and peptone serves as a nitrogen source. Therefore, the carbohydrate- and protein-rich composition of soybeans may provide nutrients that support the growth and colony formation of *Candida albicans*. In addition, lipids and minerals contribute to cellular metabolism and physiological processes that are important for fungal

development. The essential amino acid content in soy protein is strongly suspected of being able to replace peptone in SDA media as a nitrogen source for fungal protein and nucleic acid biosynthesis.

Several previous studies have shown that using legumes or grains as culture media can support optimal fungal colony growth [19]. Empirically, the use of grain or legume-based macrocomponents has been proven to successfully support the growth of colonies of several mold and yeast species both macroscopically and microscopically [20]. The Ministry of Agriculture reports that Indonesia has six superior local soybean varieties, namely Dega 1, Detap 1, Dena 1, Dering 1, Anjasmoro, and Grobogan. The soybean variety used in this study was Grobogan, a superior variety released by BALITKABI in 2008. Grobogan soybeans have a 100-seed weight of approximately 18 g and contain 43.9% protein and 18.4% fat [21]. The relatively high protein content of this variety may provide a substantial nitrogen source for fungal growth, making it a promising candidate for the development of an alternative culture medium for *Candida albicans*.

Although soybeans offer promising nutrient density, their specific effectiveness in facilitating *Candida albicans* growth kinetics needs to be tested in direct comparison with standard SDA media. Morphological variables such as colony diameter, pigmentation, surface texture, growth rate, and visualization of yeast cells and pseudohyphae must be rigorously analyzed to ensure the viability of this alternative medium [22,23].

By utilizing locally available natural resources, the development of alternative fungal culture media may be achieved in a more efficient and environmentally sustainable manner. Nevertheless, it remains unclear whether a culture medium prepared from Grobogan soybeans (*Glycine max* (L.) Merrill) can support the growth of *Candida albicans* comparably to the standard Sabouraud Dextrose Agar (SDA) medium. Therefore, this study aimed to compare the growth of *Candida albicans* on a Grobogan soybean-based medium and SDA. The results are expected to provide scientific evidence regarding the feasibility of using Grobogan soybean as an alternative substrate for fungal culture media and to expand the utilization of local biological resources in microbiological applications.

RESEARCH METHOD

This study was conducted at the Microbiology Laboratory of STIKES Hutama Abdi Husada Tulungagung in March–April 2025. The research design used in this study was an experimental design employing a post-test only control group. This design involved two groups: an experimental group that received the treatment and a control group that did not. After the treatment, both groups were tested (post-test) to compare the results. The population studied in this research was the fungus *Candida albicans*, while the samples consisted of *Candida albicans* isolates obtained from Agavi Laboratory in Bandung, West Java.

The equipment used in this study included: an analytical balance, stirring rods, beakers, measuring cups, Erlenmeyer flasks, Petri dishes, a Bunsen burner, matches, a tripod, wire mesh, sieves, a stove, an autoclave, an incubator, an oven, a horn spoon, a colony counter, a round loop, fatty cotton, wax paper, string, microscope slides, cover slips, a pH meter, test tubes, a test tube rack, micropipettes, and tips.

The materials required for this study include *Candida albicans* ATCC 14053 isolates, Grobogan variety soybeans, SDA powder, Bacteriological Agar powder, glucose powder, sterile physiological saline, $BaCl_2$, H_2SO_4, distilled water, and chloramphenicol.

Preparation of the Medium

1) Alternative medium from soybeans (*Glycine max* (L.) Merrill)

Weigh 96 grams of soybeans, 3.2 grams of glucose, and 4.8 grams of agar using an analytical balance. Place the 96 grams of soybeans into a pot. Add 320 ml of distilled water, then bring to a boil on the stove. Once boiled and softened, the mixture was strained to extract the soybean starch. The soybean starch was transferred to an Erlenmeyer flask and reheated over a Bunsen burner. 3.2 grams of sugar and 4.8 grams of agar were added to the aquous soybean starch (soybean filtrate), and the mixture was

stirred until homogeneous. The pH is measured with a pH meter to ensure it is <7.0. The mixture is poured into an Erlenmeyer flask, the mouth of the flask is covered with a cotton plug, and the flask is sealed with wrapping paper. The medium is sterilized in an autoclave at 121 °C for 15 minutes. Add 0.1 g of chloramphenicol, then shake the Erlenmeyer flask to dissolve it completely. Pour the medium into 16 Petri dishes, 15–20 ml each, under sterile conditions near a Bunsen burner flame. Allow the medium to sit in the Petri dishes until it cools and solidifies.

2) SDA Medium

A total of 20.8 g of SDA powder was weighed and dissolved in 320 mL of distilled water in an Erlenmeyer flask. The mixture was heated while continuously stirred until completely dissolved. The pH of the medium was measured using a pH meter and adjusted to below 7.0. The flask was then plugged with cotton, wrapped with paper, and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the medium was allowed to cool to approximately 45–50°C before the addition of chloramphenicol to minimize degradation of the antibiotic due to excessive heat. Chloramphenicol (0.1 g) was added aseptically to the sterile medium and mixed thoroughly until completely dissolved, resulting in a final concentration of 312.5 mg/L. The medium was then poured aseptically into 16 sterile Petri dishes (20 mL per plate) near a Bunsen burner flame and allowed to solidify before use.

Preparation of a *Candida albicans* ATCC-14053 Suspension

A McFarland 1 standard was prepared by adding 9.5 mL of H_2SO_4 and 0.5 mL of $BaCl_2$ to a test tube and mixing thoroughly. Next, approximately one-quarter of a test tube was filled with sterile physiological NaCl solution. A colony of *Candida albicans* ATCC-14053 was picked using a flamed loop and transferred into the test tube containing the sterile physiological NaCl solution. The *Candida albicans* ATCC-14053 suspension was homogenized and adjusted to a turbidity of McFarland 1; if the suspension was not turbid enough, *Candida albicans* ATCC-14053 isolate was added, and if the suspension was too turbid, sterile physiological saline was added until the turbidity matched McFarland 1.

Fungal Inoculation Stage

All inoculation procedures were performed aseptically near a Bunsen burner flame, and the work surface and equipment were disinfected before use to minimize contamination. The inoculating loop was sterilized by heating over the Bunsen burner flame until red-hot and then allowed to cool. A suspension of *Candida albicans* was collected using the sterile inoculating loop and inoculated onto the surface of the culture medium using a streaking technique. The streaking procedure was performed consistently on all plates using the same inoculation pattern to ensure uniform distribution of the fungal suspension across the medium surface. After inoculation, the Petri dishes were closed and sealed. The inoculating loop was then re-sterilized before subsequent use. All inoculated plates were incubated at 37°C for 24 h, after which colony growth was observed and the number of colonies formed on each plate was recorded.

RESULTS AND DISCUSSION

The objective of this study was to determine whether soybean-based media could serve as an alternative medium for the growth of *Candida albicans* by observing *Candida albicans* colonies on alternative media made from Grobogan soybeans and comparing their macroscopic appearance with that of *Candida albicans* colonies on SDA.

Table 1. Characteristics of Growth Media for *Candida albicans*

Fungal Growth Medium	pH	Consistency
Grobogan Soybeans	6,0	Solid
Saboraud Dextrose Agar (SDA)	6,0	Solid

Based on pH and consistency measurements of the alternative medium made from Grobogan soybeans and the SDA medium, both yielded the same results: a pH of 6 and a solid consistency.

Table 2. Number of *Candida albicans* Colonies on Growth Media

Replication	Alternative medium soybeans	Saboraud Dextrose Agar (SDA) Medium
1	198	45
2	259	15
3	172	19
4	60	12
5	88	24
6	35	29
7	49	51
8	44	22
9	107	10
10	58	19
11	48	36
12	129	44
13	103	49
14	63	28
15	95	53
16	59	39
Average	98 colony	31 colony

Based on this study, the colony counts are shown in Table 2. The average number of colonies growing on the alternative medium made from Grobogan soybeans was 98, while on SDA it was 31.

Table 3. Normality Test

Medium	Kolmogorov-Smirnov		
	Statistic	df	Sig.
Alternative Medium	.209	16	.059
SDA Medium	.129	16	.200

Based on the results of the Kolmogorov-Smirnov normality test, the Sig. value for the Soybean Alternative Medium was 0.059 > 0.05 and the Sig. value for the SDA medium was 0.200 > 0.05, so it can be concluded that the research data are normally distributed.

Table 4. Homogeneity Test

Number of Colonies	*Sig.*
Mean	0,002
Median	0,009

Based on the results of the homogeneity test, the mean was 0.002, and the median was 0.009; since these values were < 0.05, the data were found to be non-homogeneous. Based on the results of the independent t-test, the p-value was 0.001 and α was 0.05; since the p-value is less than α, H_0 is rejected, and H_1 is accepted. Therefore, it can be concluded that "There is a difference in the growth of *Candida albicans* on the alternative media of soybean (*Glycine max* (L.) Merrill) and SDA."

Table 1 shows the results of pH and consistency measurements: the alternative medium made from Grobogan soybeans had a pH of 6.0 and a solid consistency. At the same time, SDA also had a

pH of 6.0 and a solid consistency. This is consistent with the theory proposed by Jiwintarum et al. [5], who reported that *Candida albicans* grows optimally at pH 4.5–6.5. Both the alternative medium made from Grobogan soybeans and SDA fall within the pH and consistency ranges suitable for the growth of *Candida albicans*, confirming that *Candida albicans* can grow well under these conditions.

Table 2 shows that the mean number of *Candida albicans* colonies growing on the alternative medium made from Grobogan soybeans was 98. In contrast, on SDA, it was 31, according to a study conducted by Rahman et al. [16] titled "The Effectiveness of Various Concentrations of Soybean Powder (*Glycine max* (L.) Merill) as a Peptone Substitute in *Candida albicans* Growth Media," the complex nutritional content of soybeans can be utilized by *Candida albicans* for growth and development. This study aligns with this theory, as the average number of colonies growing on the alternative Grobogan soybean medium was higher than that on SDA. Based on the average number of colonies, the soybean medium had a higher average number of fungal colonies than SDA; this may be due to the high protein content of the Grobogan soybean variety, which is 43.9%. *Candida albicans* uses proteins as sources of nitrogen and carbon to build its cells by breaking them down into amino acids [24]. This confirms that soybeans can be used as a substitute for peptone in growth media for *Candida albicans*.

Based on the Kolmogorov-Smirnov normality test in Table 3, the result was Sig. > 0.05, indicating that the data are normally distributed. Based on the homogeneity test in Table 4, the result was Sig. < 0.05, indicating that the data are not homogeneous. In this study, the Independent T-test was used to compare the means of two groups that are not related on a numerical data scale. Based on Table 5, the p-value was 0.002 and $\alpha = 0.05$; since p-value < α, H_0 was rejected, and H1 accepted, meaning "There is a difference in the growth of *Candida albicans* on soybean (*Glycine max* (L.) Merrill) alternative medium compared to SDA." This aligns with the study by Rahman et al. [16], which found that *Candida albicans* colonies grew very effectively on soybean alternative media at concentrations of 5% and 7% compared to SDA. Based on the number of colonies growing on these two media, the alternative soybean medium for the Grobogan variety supported greater growth of *Candida albicans* than SDA.

Based on the data from this study, the highest number of colonies grew on SDA, at 53, while the highest number on the alternative medium made from Grobogan soybeans was 259. Differences in colony counts across media and replicate plates may be attributed to potential contamination during plate inoculation, including air, humidity, pH, and temperature variations [25]. Temperature is a critical factor in the growth of *Candida albicans*. This fungus typically grows optimally at temperatures between 30°C and 37°C. A temperature of 37°C, which is human body temperature, is the ideal condition for *Candida albicans* to multiply. The incubation period also affects the growth of *Candida albicans*. Generally, the recommended incubation time for the growth of this fungal colony ranges from 24 to 48 hours. Furthermore, due to the differing nutrient content, the average number and size of fungi that grow on the soybean alternative medium differ significantly from those on the SDA medium.

CONCLUSION

Based on the research results, alternative media made from soybeans (*Glycine max* (L.) Merrill) Grobogan variety is able to support the growth of *Candida albicans* with an average number of colonies of 98 colonies, higher than Sabouraud Dextrose Agar (SDA) media which produces an average of 31 colonies. The results of the Independent t-test showed a significance value ($p = 0.002$) which is smaller than $\alpha = 0.05$, so there is a significant difference between the growth of *Candida albicans* on alternative soybean media and SDA media. Thus, alternative media made from soybeans Grobogan variety has the potential to be used as an alternative media for the growth of *Candida albicans*.

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AUTHOR CONTRIBUTIONS

Conceptualization, FFA and EP. Methodology, QAF. Software, IPK. Validation, EP and IPK. Formal Analysis, QAL. Investigation, IPK. Resources, FFA. Data Curation, QAL. Writing Original Draft Preparation, FFA and EP. Writing Review and Editing, IPK. Visualization, QAL. Supervision, EP. Project Administration, IPK. Funding Acquisition, all authors. All authors reviewed and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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