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Peanut Sucrose for Modifications Medium on Growing of *Candida Albicans* and *Tinea Versicolor*

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Abstract

The potency of local peanut can be utilized as fungi growth medium to substitute Potato Dextrose Agar media (PDA). Peanuts are widely found in East Java and there are 7 varieties, namely Takar-1, Takar-2, Tuban, Jerapah, Talam-1, Hypoma-2, and Bima. This study aimed to make modified media from nuts for growth of *Candida albicans*, and *Tinea versicolor*. The type of this research was exploratory design. Peanut Sucrose Agar (PSA) medium was made from 8 local peanut varieties, sucrose, agar, chloramphenicol. As a gold standard media used PDA and Sabaroud Dextrose Agar (SDA). The mediums were planted with *Candida albicans*, and *Tinea versicolor*, to analyze colony diameter, morphological features and storability PSA media. The largest diameter of *Candida albicans* colonies and the highest number of *Tinea versicolor* colonies were obtained in PSA from Talam-1 variety when compared with PDA and SDA media. There was no difference in morphological of *Candida albicans* features and *Tinea versicolor* on PSA, PDA and SDA media. Storability of PSA, PDA and SDA media for 1 month at 4°C does not cause macroscopic media changes. Local peanut varieties Talam-1 was the best variety as the base material for the manufacture of PSA media for the growth of *Candida albicans* and *Tinea versicolor.*

Keywords: *Candida albicans, Tinea versicolor, Peanut sucrose agar*

Introduction

Skin disease is one of the diseases that is still a public health problem of Indonesia. The results of a survey conducted by the author on the Division of Mycology of Skin and Sex Disease Unit in Dr. Soetomo Hospital, Surabaya in 2015, skin diseases with the highest sequence of incidence. The species of dermatophyta class are *Trichophyton mentagrophytes*, *Saccharomycetes*, *Candida albicans*, and the last is *Malassezi*a with *Tinea versicolor* species.

Tinea versicolor, also known as *Pityriasis versicolor* or fungus, is a superficial fungal infection of the skin caused by *Malassezia furfur* and is characterized by a skin macula, a mild and itchy scale, this infection is chronic, mild and usually without inflammation. *Tinea versicolor* more frequently concerns the face, neck, torso, upper arm, armpits, thighs and groin[(1)].

Symptoms of fungal infections are usually arising the spots either white, brown or red, depending on skin color, palpable like fine scales. Scales that when scratched, will come out small white and powder-like grains, when it is sweating it will feel very itchy. As a lipophilic organism, *Malassezia furfur* requires lipids for in vitro growth and in vivo[(2)].

Candida sp is known as a dimorphic fungus that normally exists in the gastrointestinal tract, upper respiratory tract and genital mucosa in mammals[(3)], but it can cause infection problems that in increasing population when the immune system declines both locally and systemically[(4)].

One of the procedures in the laboratory to support the diagnosis of *Candida* is to use the seed medium / culture. In seed medium have requirement of nutritional adequacy, temperature and pH according to requirement of microorganisms to be breed because there is different requirement of nutrition depend on type of

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microorganism, but basically it have the same basic requirement, that is water, carbon, energy, mineral[5],[6]. The standard media for growing fungi is Potato Dextrose Agar media (PDA) and Sabaroud Dextrose Agar (SDA). PDA and SDA media contain carbohydrates in potatoes as nutrients and dextrose which is an additional material that is a source of carbon for mold growth.

Peanuts grown by several farmers in East Java are 7 kinds of varieties: Takar 1, Takar 2, Tuban, Jerapah, Talam 1, Hypoma 2, and Bima. Preliminary studies conducted by Fifi[8] suggest that modified media made from peanuts or Peanut Sucrose Agar (PSA) can be used as a substitute for PDA media for the growth of fungi such as *Trichophyton mentagrophytes*. Peanut concentrations in PSA media in the Fifi study showed optimal colony growth was 300 mg / 500 mL.

Local peanut varieties as the base material for PSA media which shows the best growth of Trichophyton mentagrophytes colony is Takar 2. The economical side of making Peanut Sucrose Agar medium to be cheaper than the other standard media (51,7% from media manufacture PDA and 71.2% of SDA production)[8]. For *Saccharomycetes* and *Malassezia* class fungi (the most fungal cause of skin diseases according to data in Dr Soetomo Hospital, Surabaya), the effectiveness of the use of PSA media has not been tested. The objective of this research was to analyze the growth of *Candida albicans* colon and *Tinea versicolor* on Peanut Sucrose Agar modified medium and PSA media resistance during storage at 4ºC.

Method

The type of this research is exploratory, the samples used are peanuts in East Java, namely: Takar 1, Takar 2, Tuban, Talam 1, Hypoma 2, Jerapah, Gajah and Bima, taken with random allocation and *Candida albicans* and *Tinea versicolor* obtained from Dr Soetomo Hospital, Surabaya. Peanuts

Materials

Candida albicans and *Tinea versicolor*, groundnuts with certain varieties, sucrose or sugar, bacteriological agar, chloramphenicol, Methylen Blue dyes, Potato Dextrose Agar as well as aquadest, inoculum hooks, autoclave, fatty cotton, analytical scales, petri dishes, erlenmeyer, bunsen, foot three, wire mesh, funnel, glass object, glass cover, microscope, balance, measuring pipettes, volume pipettes, and beaker glass.

Preparation of Potato Dextrose Agar (PDA) medium as Gold Standard (Neogen corp, Catalogue no: 7149)

The 19.5 grams PDA media powder, dissolve in aquadest as much as 500 mL into erlenmeyer. The PDA solution is heated to homogeneous and boils over the hot plate. The pH of the media was adjusted to pH 5.6, then sterilized in an autoclave at 121°C for 15 minutes. Chloramphenicol is added at a dose of 10 mg/ 100 mL. The media is poured in petridish and cooled at room temperature.

Preparation of Media Modification Peanut Sucrose Agar

The 300 grams Peanuts are mashed and blended without water and then boiled in 500 ml of aquades for 15 minutes. The filtrate was added 20 grams of sucrose, 10 grams of agar and some of the aquades until the final volume of 1000 ml was obtained and heated. The pH medium was adjusted to 5.6 and the media was sterilized in an autoclave at 121 ° C, for 15 minutes. Chloramphenicol is added with a dose of 10 mg / 100 mL and then the media is poured in petridish and cooled to room temperature[8]

Preparation of Sabouraud Dextrose agar media (SDA)

The 65 grams SDA powder are dissolved in 1000 ml aquadest, then measured its pH 5.6 ± 0.2, sterilization with autoclave at 121 °C for 15 minutes. Chloramphenicol is added with a dose of 10 mg / 100 mL, then distributed to petridish.

Breeding of *Candida albicans* and *Tinea versicolor*

Inoculated *Candida albicans* and *Tinea versicolor* cultures on modified Peanut Sucrose agar medium and PDA media, then incubated at 25°C for about 2 weeks

Identification of *Candida albicans* and *Tinea versicolor*

The object glass is prepared and then stained with a Methylen Blue dye of about one to two drops. The colony is taken using the inoculum hook of each medium and mixed with the Methylen Blue dye. Closes with cover glass then fixes two to three times. Observe on a 10x magnification microscope to look for a field of view and 40x to clarify the structure of the mold morphology.

PSA Media Resilience During Storage at 4ºC

The resistance of PSA media during storage at 4 ºC is a media quality test that includes visual tests and sterility tests. Test visually by observing the change of color, turbidity, and PSA media quality visually compared to PDA and SDA media stored at the same temperature and time. The sterility test is performed by incubating the media for 2-3 days in the incubator

Findings

The Growth of *Tinea versicolor* on media

The Results of Microscopic Observation

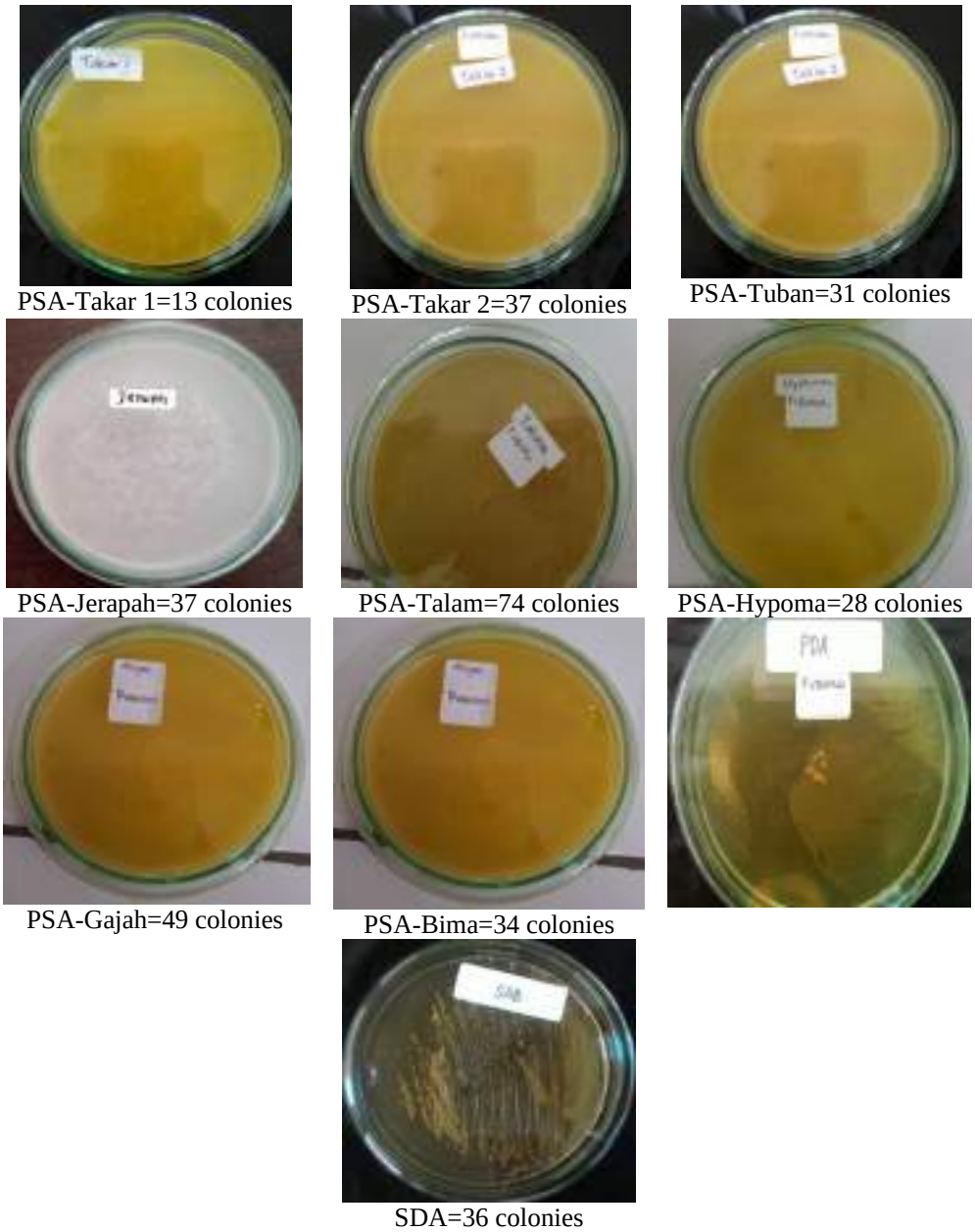


Figure 1. The Fungus Growth of *Tinea versicolor* on media (incubation at 25°C for 2 weeks)

The Growth of *Candida albicans* on Media

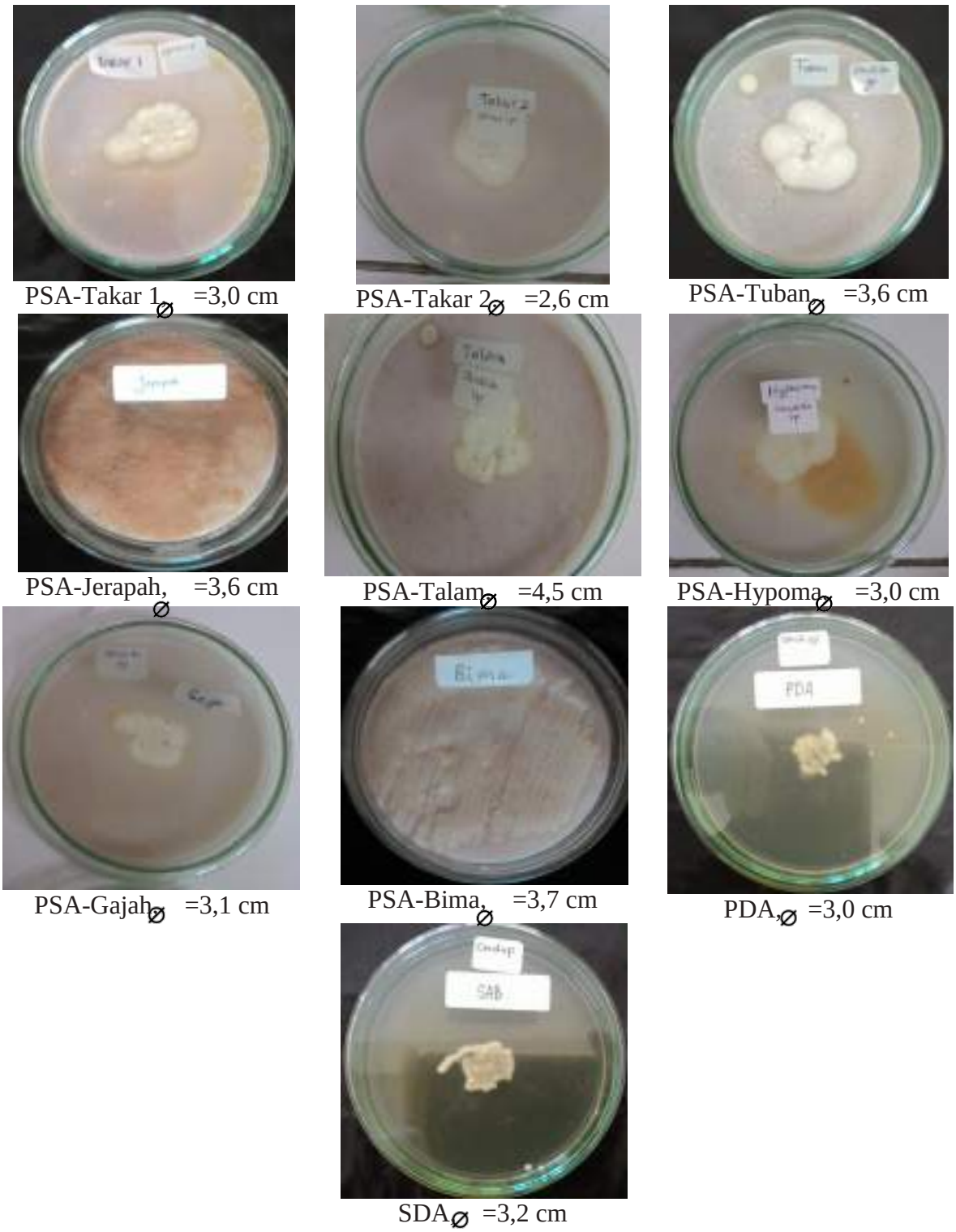
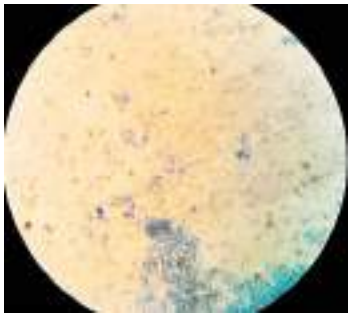


Figure 2. The Fungus Growth of *Candida albicans* on media (incubation at 25°C for 2 weeks)

Figure 3. The results of microscopic observation



Tinea versicolor

Candida albicans

Table 1. The results of durability test in storage for 1 month at 4 °C on PSA media

No	PSA media with Peanut varieties	1st week	2nd week	3rd week	4th week
1	PSA Takar 1	-	-	-	-
2	PSA Takar 2	-	-	-	-
3	PSA Tuban	-	-	-	-
4	PSA Jerapah	-	-	-	-
5	PSA Talam	-	-	-	-
6	PSA Hypoma 2	-	-	-	-
7	PSA Gajah	-	-	-	-
8	PSA Bima	-	-	-	-
9	*Potato Dextrose Agar*	-	-	-	-
10	*Sabouraud Dextrose Agar*	-	-	-	-

(+) Be changes in the texture of the media

(-) No changes in the texture of media (sterile)

Discussion

Of the 8 varieties of peanut (Takar 1, Takar 2, Tuban, Jerapah, Hypoma 2, Talam, Gajah and Bima) as the basic ingredients of making PSA obtained the result:

a. The largest diameter colonies of Candida albicans fungi were found on PSA media from peanut varieties of Talam with size 4.5 cm. The size of the colony was larger than that of Sabouraud Dextrose Agar (3.2 cm) and Potato Dextrose Agar (3.0 cm) as gold standard medium

b. The highest number of Tinea versicolor mushroom colonies was obtained on PSA media from peanut varieties of Talam of 74 colonies. The number of colonies is greater than the media Sabouraud Dextrose Agar and Potato Dextrose Agar as gold standard media

Nutrient quality related to nutrient composition in culture medium and water quality parameters[6]. From research conducted by Retno[8], obtained data of carbohydrate, protein and fat content of Potato Dextrose agar media to have the following composition: 3.67; 3.68 and 1.69%. The content of carbohydrates, proteins and

fats on the PSA medium of peanut varieties of Talam 1 has the following composition: 3.44; 3.08; and 1.28%. If the composition of the media is compared, it appears that the nutrient content contained in the PSA is lower than the PDA, but the diameter of Candida albicans colony is larger in the PSA medium.

Although PSA nutrient content is lower than PDA but the nutritional composition of PSA media is the optimal composition for Candida albicans colony growth. Carbohydrates are composed of 3 types of elements, namely carbon, hydrogen and oxygen. Which include carbohydrate compounds are sugar, starch and cellulose. Fungi depend on the complex carbohydrates as a nutrient source. Carbohydrates are described first into monosaccharide form with extracellular enzymes then newly absorbed by fungi for further assimilation. Carbon sources are needed for the energy and structural needs of fungal cells. This supports the growth of mycelium in the medium[12]. Candida albicans requires organic compounds as a source of carbon and energy sources for their growth and metabolic processes. These carbon elements can be obtained from carbohydrates. Draski[7] research on the influence of media type and phosphorus composition on the growth of white oyster mushroom indicates that the type and composition of the media influence the growth of fungus.

In addition to mushrooms, peanut and other dairy products are good ingredients for growth of *Salmonella sp*[5], *Bacillus sp, Proteus sp, Staphylococcus sp, Escherichia coli*, and *Serratia sp*[10]. This suggests that nuts are a good growth medium for bacteria and fungi.

The macroscopic morphology of *Candida albicans* on PSA media made from 8 peanut varieties has no significant difference, having a rounded shape with a slightly convex, smooth, slippery surface and sometimes a few folds especially in old colonies. Age of culture affects large small colonies. Colonies of yellowish-white color and smelled sour like tape scent. Candida albicans can grow on a wide variety of pH, but its growth will be better at pH between 4.5-6.5. This fungus can grow in seedling at a temperature of 28ºC - 37ºC. Candida albicans has three forms of microscopic morphology:

1. Yeast Like cells, seen as a collection of round or oval cells with variations of 2-8 µm wide and 3-4 µm in length, 1.5-5 µm in diameter. These cells can form blastospores.

2. Pseudohypha, because the blastospores do not escape and continue to form new shoots.

3. Chlamydospores, round cell walls with a diameter of 8-12 µm. Chlamydospores are formed when Candida albicans is cultured in less nutrient medium such as Corn meal agar.

Microscopic morphology of Candida albicans on PSA media made from 8 peanut varieties has no significant difference. The type of microscopic morphology seen in Candida albicans grown in PSA from 8 peanut varieties is the Yeast Like cells type.

The physical structure of Candida albicans consists of cell walls, cell membranes, cytoplasm and nucleus. Candida albicans cell membrane consists of double phospholipid (lipid bilayer), the outer layer is rich in phosphatidyl, choline, ergosterol and sphingolipids. Sphingolipids contain the largest negative component of the plasma membrane and play an important role as an antimicrobial target[9].

The macroscopic morphology of Tinea versicolor on PSA media made from 8 peanut varieties has no significant difference. The incubation takes 1-2 weeks, the colony grows like yeast, is green and at the edges grows a filamentous area of brown.

The microscopic morphological features of Tinea versicolor on PSA media made from 8 peanut varieties have no significant difference, in the form of groups of round cells, sprouted, thick-walled and have short-stemmed and bent hyphae, smooth branched, shiny and spores oval.

Storage in the refrigerator at 4 °C for 1 month, with weekly observation, was not found to be change in PSA media, it also happened on Potato Dextrose agar media and Sabouraud Dextrose Agar. This indicates that PSA media have the same storage power as WHO standard media used as gold standard medium.

Additional Informations

Source of Funding: Authors

Conflict of Interest: No

Ethical Clearance: Yes

Conclusion

From the results of research that has been implemented it can be concluded as follows:

1) The best varieties of peanuts as the base material for the manufacture of PSA media with the growth of Candida albicans and Tinea versicolor are Talam-1, because the growth of fungi indicates better diameter and colony amount compared to PDA media and Sabouraud Dextrose Agar as gold standard media.

2) Macroscopic and microscopic morphology of Candida albicans and Tinea versicolor in some peanut varieties in PSA has no difference when compared with the growth of fungus on PDA media and Sabouraud Dextrose Agar as gold standard media.

3) PSA media stored for 1 month at 4oC did not change and the same result also happened on PDA media and Sabouraud Dextrose Agar as gold standard media

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