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Colony Morphology of Yeast Isolates from Tuak and Its Application in Producing Ethanol From Sugarcane Bagasse

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Abstract: Colony morphology yeast isolates from tuak and its application in producing ethanol from sugarcane bagasse has been conducted. Yeast isolates used in this work were HT4, HT5, and HT20 obtained from Tuak. These isolates have cream to yellow in color, spherical to oval in shape, and grow as budding cells. Our goal is to find yeast isolates used as microbial agent in fermentation process producing ethanol from lignocellulosic biomass as raw material. Based upon morphology showed that these isolates were *Candida* species. Isolate HT5 then used to see its potent as agent in bioethanol production. Fermentation of 5 g sugarcane bagasse substrate resulted 0.0008% ethanol. This results indicated that isolate has a potent as microbial agent for fermentation, however the optimum condition of process is needed to furthermore study.

Keywords: Candida species, bioethanol, Tuak

Abstrak (Indonesian): Telah dilakukan penelitian terhadap morfologi koloni dari isolat khamir dari tuak dan aplikasinya dalam produksi etanol dari ampas tebu. Isolat khamir yang digunakan adalah koloni HT4, HT5, dan HT20 yang telah didapat dari tuak. Isolat-isolat ini memiliki bentuk warna krem hingga kuning dengan bentuk bulat hingga oval dan pertumbuhannya dengan membentuk tunas. Tujuannya adalah untuk mendapatkan isolat ini akan digunakan sebagai agen mikroba pada proses fermentasi menghasilkan etanol dari biomasa lignoselulosa sebagai bahan bakunya. Berdasarkan morfologi menunjukan bahwa isolat-isolat ini adalah spesies *Candida*. Isolat HT5 selanjutnya digunakan untuk melihat potensinya sebagai agen dalam produksi bioetanol. Fermentasi menggunakan 5 g subtrat ampas tebu menghasilkan etanol sebesar 0.0008%. Hasil ini mengindikasikan bahwa isolat memiliki potensi sebagai agen fermentasi menghasilkan etanol akan tetapi masih perlu diteliti lebih lanjut untuk kondisi optimum prosesnya.

Kata kunci: spesies Candida, bioetanol. tuak

1. Introduction

Microorganism plays an essential role in natural reactions. Microorganism primary found in foods from several sources such as soil, water, air, during processing, transportation, and storage of food. Some food associated microorganism due to the availability of nutritional requirement and the environmental factors. Fermentation alcohol is a process that converts sugar into alcohol using microorganism. Although *Saccharomyces cerevisiae* has a central position among bioethanol-producing organism [1], however other yeast species or bacteria are be able to convert sugar into ethanol. Bioethanol production using *S.cerevisiae* with different perspectives like substrate, growth variables, inhibitor reduction, and immobilization was studied [2]. *Zymomonas mobilis* yields high ethanol and can tolerate high ethanol concentration [3], Advanced process techniques used forethanol production are reviewed with an emphasis on the advantages of using thermophilic bacteria [4]; Bioethanol production by recycled

Scheffersomyces stipites in sequential batch fermentations with high cell density using xylose and glucose mixture [5]. Yeast species has been isolated from Tuak , a fermented drink. Tuak, a traditional beverage is produced by spontaneous fermentation of the palm sap (*Arenga pinnata*) in the presence of raru wood for overnight incubation [6]. Our goals is to find out microbe agent for intentional use of fermentation to produce bioethanol using raw material lignocellulosic biomass. Morphologically characterization is one prominent observation in order to purify and to identify yeast isolates. These isolates had cream to yellow in color, spherical to oval in shape, and grow as budding cells. Based upon morphology expected that yeast isolates are Candida species

2. Experimental Sections

Isolates, strains, and media

Yeast species isolates HT4, HT5, and HT20 was selected from filtered fresh Tuak spreaded on YPDA agar medium with 100 ug/mL chloramphenicol, while as comparison we used *S.cerevisiae* laboratory strain BY4741 with genotype *MATa met15Δ0 his3Δ1 leu2Δ0 ura3Δ0* (Htg$^-$). YPDA broth medium consisted of 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 400 mg/L adenine, while YPDA agar medium added 20 g/L bacto agar).

Culture

Routine work for cultivation, isolates and yeast strain were grown by streaking way on YPDA agar media plate to reach single colonies, and then incubated at 30^{o}C for 1-2 days.

Spot culture

5 mL cells grown on YPDA broth culture until reach OD_{660} 1.0, then cells were collected using centrifuge 5000 rpm, and resuspended cell with a sterile water adjusted until getting cells with concentration 10^6 cells/mL. Using micropipette, 5 µL cell suspension spotted on YPDA agar media plate. Incubated the cells at 30^{o}C for 1-2 day.

Microscopic observation

5 mL cells grown on YPDA broth culture until reach exponential phase or OD_{660} 1.0, then cells were collected using centrifuge 5000 rpm, and resuspended cell with 1 mL sterile water. One drop of immersion oil was added into 5 µL cells on glass, and cells the observed using Microscope (Olympus).

Fermentation test

Isolate HT5 added into 50 mL culture containing 10% sugarcane bagasse, incubate with shaking at room temperature for 2-3 days. Ethanol produced in this ferementation was analyzed using Gas Chromatography Shimadzu 5810.

3. Results and Discussion

Isolates HT4, HT5, and HT20 were obtained from purification isolates from tuak. These isolates were streaked on YPD medium to get single colonies. Under standard conditions at temperature 30^{o}C incubated for 1-2 days with nutrients got from YPDA medium, hundreds of single colonies HT4, HT5, and HT20 appear after within one day incubation. The characteristic of cells were cream to yellow in color, smooth in texture, and listening as shown in Figure 1. Macroscopically, yeast *S.cerevisiae* on the routinely used YPDA medium, while other yeast like *Candida* species used Sabouraud dextrose agar consisting of 40 g/L dextrose, 10 g/L peptone, and 15 g/L bacto agar for agar medium and without bacto agar added for broth medium [7]. For the *Candida* species it is depending on the species, colonies *Candida* are cream to yellow, colony texture may be smooth, glistening or dry, or wrinkled and dull [8].

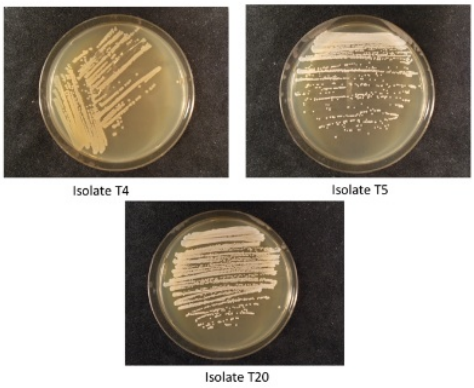

Figure 1. Shape of isolate HT4, HT5, and HT20 colonies

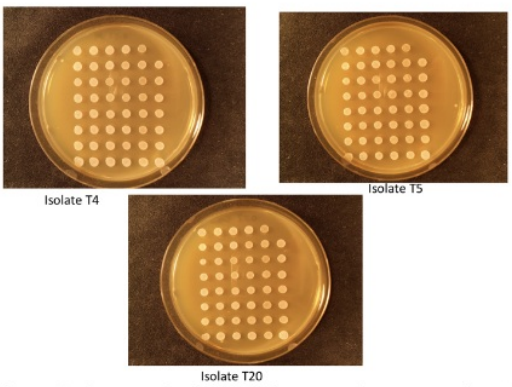

Figure 2. Spot analysis of isolates HT4, HT5, and HT20 colonies

Furthermore, in order to observe rate of growth, we incubate 5 µL 10^6 cells/mL of cells suspension each 36 single colonies of HT4, HT5, and HT20 at 30^{o}C spotted on YPDA agar media plate, within 18 hours these colonies have clearly been seen as shown in Figure 2.

This indicated that these colonies grow easily in appropriate media since it contains dextrose and peptone. To see the morphology, isolates at exponential phase in growth were observed as shown at Figure 3. Single cells in unbudding and unbudding cells form with various size of daughter cell before separate as a new single unbdding cells was observed. Shape of cells isolates HT4, HT5, and HT20 were spherical to oval. When the Candida species grow in appropriate nutrient in log phase as budding (blastoconidia) which is approximately 2-5 x 3-7 µm, or 4–8 × 5–11 µm in size [9]. Silva et al [8] reported that *Candida* species can produce a filamentaous type of growth, pseudohypae or true hypae, or The distinction between hyphae and pseudohyphae is related to the way in which they are formed. Pseudohyphae are formed from yeast cells or hyphae by budding. Based upon above observation HT4, HT5, and HT20 is *Candida* species.

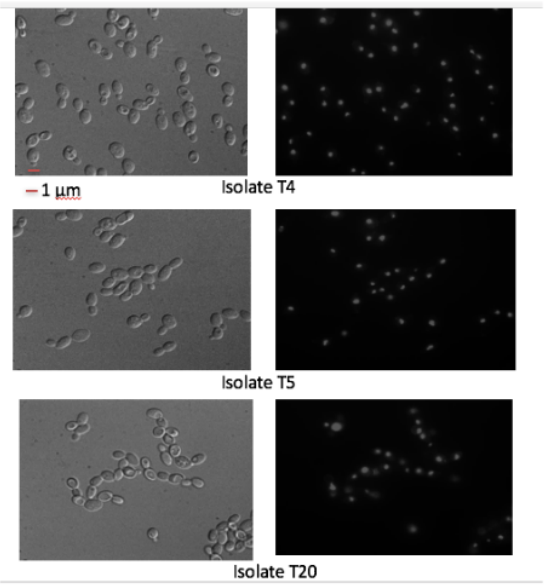

Figure 3. Morphology of isolates HT4, HT5, and HT20 colonies

Then, to see whether this *Candida* isolates was potent to be microbe agent for bioethanol production, fermentation of sugarcane bagasse was applied in this research. Sugarcane bagasse is one of lignocellulosic biomass potential that can be converted into bioethanol. Using only the isolate HT5, we ferment a thermal-treated sugarcane bagasse, and using gas chromatography it produced 0.0008 % ethanol with retention time 1.617 as shown in Figure 4. However this fermentation process produced very low concentration, we need to optimize the process. Since, previous report using *C.tropicalis* in the presence of exogenous alpha-amylase, 9% (w/v) soluble starch was converted to 43.1g ethanol/l in 65 h with a productivity of 0.65 g/l [10]. Thus, Candida species has a potent to be microbial agent for fermentation process which can be applied in the renewable energy production.

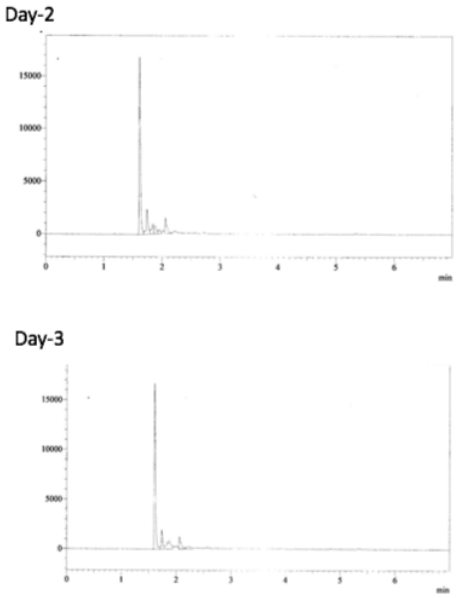

Figure 4. Chromatogram of Gas Chromatography analysis of isolates HT4, HT5, and HT20

4. Conclusion

Based on morphology, our work has been successful isolating yeast HT4, HT5, and HT20 from Tuak designated as Candida species.

In this experiment conversion sugarcane bagasse into bioethanol producing low concentration 0.0008%, but isolate has indicate as potential microbe agent in bioethanol production from lignocellulosic biomass, however optimization condition of process was needed.

References

[1] Petrovic, U., 2015, Next-generation biofuels : a new challenge for yeast, *Yeast* 32(9): 583-93

[2] Bhadana and Chauhan, 2016., Bioethanol production using Saccharomyces cerevsiiae with different perspectives:substrates, growth variables, inhibitor reduction, and immobilization, *Ferment. Technol*., 5:2.

[3] Tiwari, K.L., and Jadhav, S.K., 2011, Studies of bioethanol from some carbohydrate sources by gram positive bacteria, *Journal of Sustainable Energy and Environment*, 2:141-144.

[4] Scully, S.M., and Orlygsson, 2015, Recent Advances in Second Generation Ethanol Production by Thermophilic Bacteria, *Energies*, 8;1-30.

[5] Santos, S.C., de Sousa, A.S., Dionisio, S.R., Tramontina, R., Ruler, R., Squina, F.M., Vaz Rossell, C.E., da Costa, A.C., Lenczak, J.L., 2016, Bioethanol production by recycled Scheffersomyces stipites in sequential batch fermenattions with high cell density using xylose and glucose mixture, *Bioresour. Technol.* , 219: 319-29.

[6] Tamang, J.P., Editor. 2016, Ethnic fermented foods and alcoholic beverages of Asia, Springer. ISBN 978-81-322-2798-4.

[7] Murray, M.P., Zinchuk, R., Larone, D.H., 2005, Chrom agar Candida as the sole primary medium for isolation of yeasts and as a source medium for the rapid-assimilation of trehalose test, *J.Clin. Microbiol*., 43(3): 1210-2.

[8] Silva, S., Negri, M., Henriques, M., Oliveira, R., Williams, D.W., and Azeredo, J., 2012, Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance, *FEMS Microbiol.Rev*., 36 (2); 288-305.

[9] Larone, D., 2002, Medically Important Fungi; A Guide to Identification. 4th edn. ASM Press, Washington.

[10] Jamal,L., Ettayebi, K., El Yamani, J., Ettayebi, M., 2007, Production of ethanol from starch by free and immobilized Candida tropicalis in the presence of alpha-amylase, *Bioresour Technol.,* 98(14): 2765-70.

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