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Bioethanol Production from Cellulose by *Candida Tropicalis*, as an Alternative Microbial Agent to Produce Ethanol from Lignocellulosic Biomass

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Abstract: *Candida tropicalis* isolated from Tuak is a potentially useful microorganism for the ethanol production from lignocellulosic biomass and it can be alternative agent replacing *Saccharomyces cerevisae* for fermentation process. Although *C. tropicalis* could not convert all carbohydrates content of lignocellulosic into bioethanol, however it is able to grow on medium in the presence of either xylose or arabinose as carbon source. Our result showed that fermentation of 10 % (w/v) cellulosic as sole carbon source produced 2.88% (v/v) ethanol by *C. tropicalis*. This ethanol production was lower than usage of 10% (w/v) dextrose as sole carbon source medium which producing 5.51% (v/v) ethanol. Based upon our experiment indicated that *C. tropicalis* is able to conduct two main process in converting of cellulosic material- to ethanol which is hydrolysis the degradation of cellulose into glucose, and fermentation the process the conversion glucose into bioethanol.
Keywords: Candida tropicalis, bioethanol, fermentation, cellulosic

Abstrak (Indonesian): *Candida tropicalis* yang diisolasi dari Tuak adalah agen yang berpotensi dalam produksi etanol dari biomassa lignoselulosa dan dapat dijadikan agen alternatif menggantikan *Saccharomyces cerevisiae* pada proses fermentasi. Walaupun *C. tropicalis* tidak dapat mengonversi semua kandungan karbohidrat lignoselulosa menjadi etanol, akan tetapi *C. tropicalis* mampu tumbuh pada media dengan xilosa atau arabinosa sebagai sumber karbon. Hasil kami menunjukkan bahwa dengan menggunakan *C. ropicalis* fermentasi 10% (w/v) selulosa sebagai satu-satunya sumber karbon menghasilkan 2,88% (v/v) etanol, Produksi etanol ini lebih rendah jika menggunakan 10% (w/v) dekstrosa sebagai satu satunya sumber karbon yang menghasilkan 5,51% (v/v) etanol. Berdasarkan percobaan menunjukkan bahwa *C. tropicalis* mampu melakukan dua proses utama dalam mengonversi material selulosa menjadi etanol yaitu hidrolisis degradasi selulosa menjadi glukosa, dan fermentasi proses konversi glukosa menjadi bioetanol.
Keywords: Candida tropicalis, bioetanol, fermentasi, selulosa

1. Introduction

Renewable energy is absolutely needed in order to anticipate depletion of fossil fuel stock because demand of human energy increased. Bioethanol is a potential energy for the future, not only abundant of raw material but also clean environment, because of ethanol combustion does not produce any pollutions. Utilization of ethanol replacing fossil fuel significantly reduces carbon dioxide emission since ethanol is part of global carbon cycle, and it could be produced by fermentation process. Ethanol previously has been known as anti-knocking additive substance which added into gasoline since 1925 and it increased machinery efficiency and function of ethanol [1].

The first generation, bioethanol production utilized starch or grains, and for the second generation, raw material was lignocellulosic biomass such as rice hull, wheat husk, sugarcane bagasse, palm empty fruit bunch, corn stover, grasses and other household wastes. Thus, Lignocellulosic biomass is a promising alternative source of energy production because of a national abundance of renewable and sustainable feedstock. Lignocellulosic biomass is comprised of 42-50% cellulose (insoluble fibres $\beta-1,4$-glucan), 25-30% hemicellulose (non cellulosic polysaccharides including xylans, mannans, and glucans), lignin (a complex polyphenolic structure), and 5-8% extractive [2]. Bioconversion of lignocellulosic biomass to ethanol is environmentally friendly treatment of the most abundant lignocellulosic waste materials and alternative for sustainable green energy production.

Three main common steps in second generation bioethanol production as follows 1) Pretreatment of lignin residue, 2) cellulosic and hemicellusic hydrolysis, 3) sugar fermentation. Pretreatment process aims to separate lignin and break the structure of lignocellulose, and it is one of the most expensive steps in the process of converting biomass [3] and lignin is non fermentable material. Some hydrolysis methods producing a fermentable monosaccharide from celluloses or hemicellulose have been developed. In fermentation reaction, one sugar molecule converted to two ethanol and two carbon dioxides molecules.

Yeast *Saccharomyces cerevisiae* is common microbial agent in fermentation converting sugar to ethanol. However, *S.cerevisiae* wild type is not be able to ferment pentose D-xylose and L-arabinose, major monosaccharide in lignocellulose [4]. Utilization of lignocellulosic biomass as raw material should convert entire monosaccharides content to get optimum concentration of ethanol. The primary difficulty for commercialization of ethanol. produced by fermentation is its high cost of production comparing than gasoline production cost [5], therefore two simultaneous hydrolysis and fermentation could reduce production cost. Hence, exploration of other potential microorganism should be conducted. One of promising microbial agent for bioethanol fermentation from lignocellulosic material is Candida tropicalis isolated from Tuak [6]. *C.tropicalis* was known to produce ethanol from starch in low rate [7], however further experiments states that C.tropicalis is a promising agent to produce ethanol from renewable sources [8,9]. The advantage of using *C.tropicalis* in starch fermentation to ethanol is the process is unnecessary to perform saccharification step and the process resulting ethanol around 56 g/L. This produced ethanol is similar to fermentation using recombinant *S.cerevisiae* which express amylase and glucoamylase [7]. In this article shows us that yeast *C.tropicalis* has capability to bioconvert cellulose to ethanol without saccharification step.

2. Experimental Sections

2.1. Material and strains

C.tropicalis yeast isolated from Tuak. YPD Broth media composed of 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose autoclaved at 121^{o}C, 15 psi for 15 minutes. YPD10 media composed of 10 g/L yeast extract, 20 g/L peptone, 100 g/L dextrose. YPC10 media composed of 10 g/L yeast extract, 20 g/L peptone, and 100 g/L cellulose, YPX10 media composed of 10 g/L yeast extract, 20 g/L peptone, and 100 g/L xylose, YPA10 media composed of 10 g/L yeast extract, 20 g/L peptone, and 100 g/L arabinose.

2.3. Phenotype test

Phenotype test of yeast strain was carried out following a modified previous procedure [10]. Cells were streaked on YPD media to get single colonies, and then single colonies were streaked on YPD media with the carbon source either of 2% glucose, 2% xylose, or 2% galactose and incubated on room temperature for 1-2 days.

2.3. Ferments

C.torpicalis isolate or *S.cerevisiae* BY4741 cells were cultured in YPD medium room temperature for overnight, and these cells were transferred to fermentation medium YPC10, with initial cell density was adjusted to OD_{600} = 0.1. Fermentation was performed using culture tubes (18x175 mm) with working volume of 10 ml YPD10, YPC10, YPX10, and YPA10. medium at room temperature incubation for 1-2 days. The bioethanol concentration from fermentations were determined by using Gas Chromatography Shimadzu 5810 plus with operational condition as follows: Temperature of Column 110^{o}C, temperature of FID detector 240 ^{o}C, temperature of injection port 200^{o}C, rate of nitrogen carrier gas 50 ml/min.

3. Result and Discussion

In previous research showed that *C. tropicalis* has advantage in bioethanol production fermentation such as having higher growth rate than *S. cerevisiae*, able to grow on arabinose or xylose as sole carbon source [6]. Cellulose is one of the most important component in lignocellulosic biomass, and could be converted to ethanol. Fermentation for 1 day showed that *C.tropicalis* is capable to hydrolyze and ferment cellulose to bioethanol as shown on Fig.1a. Based on our GC condition, ethanol peak had retention time 1.98 min. Ethanol concentration produced by fermentation using cellulose as sole carbon source was lower than using dextrose as sole carbon source. For one-day fermentation process using YPC10 or 10% (w/v) cellulose containing medium produced 2.88% or 28.8 g/L ethanol, while using YPD10 or 10% (w/v) dextrose containing medium produced 5.51% (w/v) ethanol or 55.1 g/L (Fig. 1b).

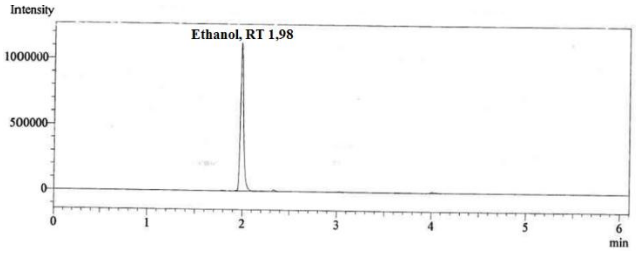

Figure 1a. Qualitative and Quantitative Analysis of ethanol production from cellulose containing medium

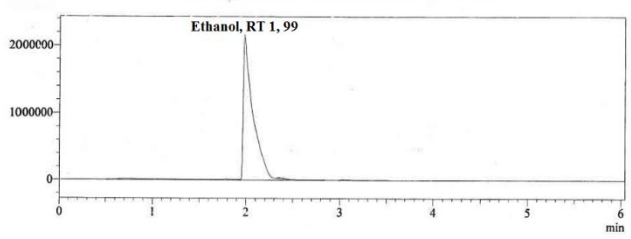

Figure 1b. Qualitative and Quantitative Analysis of ethanol production from dextrose containing medium

Based upon these results indicated that *C. tropicalis* was able to hydrolyze cellulose to glucose and then finally convert to ethanol. If 2,88 % w/v or 28.8 g/L ethanol was produced, and its stoichiometry means this reaction using 56.35 g glucose. While YPC10 medium containing 100 g cellulose per litre. This data indicated that hydrolysis using *C. tropicalis* resulted more than 50% glucose product. Cellulose is homopolysaccharides comprised of β-D-glucopyranose with β-glycosidic bond, and cellobiose is the smallest repetition unit from cellulose dan it can be converted into glucose rsidue [2]. Three groups enzymes play a role in hydrolyzing cellulose to glucose endoglucanase, cellobiohydrolase (exoglucanase), and β-glucasidase. To furthermore study, it is needed to carry out further experiments what kind of enzymes play a role in this hydrolysis process.

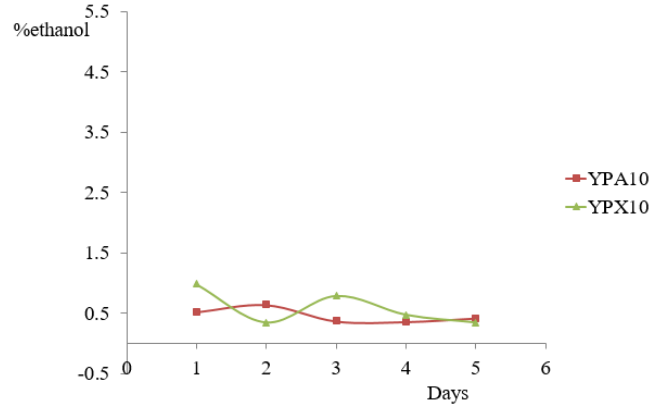

Figure 2a. Ethanol production in the presence of xylose and arabinose

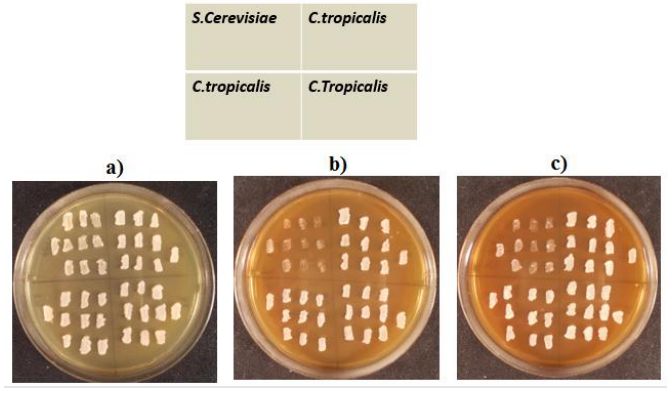

Figure 2b. Phenotype *tests C.tropicalis* on a) YPD media; b) Xylose meium; c) arabinose medium

Fermentation using YPA10 or YPX10 media in the presence of 10% (w/v) arabinose and 10% (w/v) xylose as sole carbon source respectively incubation until 5 days showed no ethanol product (Fig. 2a). On other hand, phenotype test to several *C. tropicalis* colonies showed that all single colonies grew well on arabinose and xylose media, while *S.cerevisiae* did not (Fig. 2b). These data indicated that both arabinose and xylose are utilized as carbon source for growth, but they cannot be converted to ethanol, therefore we propose a model how C.tropicalis bioconverting cellulose to ethanol as shown in Fig. 3.

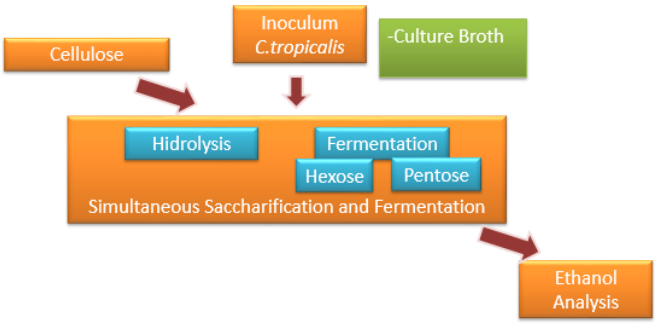

Figure 3. Model Bioconverting cellulose to ethanol by *C.tropicalis.*

4. Conclusion

C.tropicalis isolate is capable of hydolyzing and fermenting cellulose to produce bioethanol. Bioethanol is produced by bioconverting through metabolizable monosaccharide glucose, but not for monosachharides pentose such as arabinose and xylose. As major component of lignocellulosic material, this result indicates that *C.tropicalis* could be applied as microbial agent for bioconverting lignocellulosi biomass to ethanol.

Acknowledgement

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