

.33_ Arabinose and Xylose Fermentation to produce ethanol by isolated yeasts from durian (*durio zibethinus* L) fruit

By Herman syah

Bioethanol Production from Fermentation of Xylose and Arabinose Using Isolated Yeast of Durian (*Durio zibethinus* L.) FruitHermansyah^{1*}, Fachrijal¹, Miksusanti¹, Fatma¹, Getari Kasmianti², and Almunadi T. Panagan¹¹Chemistry Department, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Palembang, Indonesia²Environmental Management Study Program, Graduate School Sriwijaya University, Palembang, Indonesia*Corresponding author email: hermansyah@unsri.ac.id

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ABSTRACT. Xylose and arabinose are pentose sugars that present in hemicellulose, part of lignocellulose biomass. These pentose sugars can be fermented by yeast into ethanol. The aim of this research was to utilize yeast isolated from durian fruit (*Durio zibethinus* L.) in fermentation of xylose and arabinose to produce bioethanol. Yeast isolation was used phenotypic test. Phenotypic test of isolates was conducted by growing the isolates in various agar media, i.e. YPD (Yeast Peptone Dextrose), YPA (Yeast Peptone Arabinose), and YPX (Yeast Peptone Xylose) containing dextrose, arabinose, xylose, respectively, as sole carbon source to see cell growth. The yeast isolates were further identified using API AOC 20C kit method. Yeast isolates were applied for fermentation of glucose, arabinose, and xylose in incubated cultures. Ethanol production in the fermentation was analyzed by gas chromatography. Yeast isolates were identified as *Kodamaea ohmeri*, *Candida famata*, *Candida guilliermondii*, and *Cryptococcus laurentii*. Based on gas chromatography data, it was found that ethanol produced in the fermentation for three days, the highest ethanol content on xylose substrate was fermented by *Candida famata*-A which is 0.021% (v/v) ethanol resulted from initial concentration of 5% xylose (w/v). While on arabinose substrate, the highest ethanol content was fermented by *Cryptococcus laurentii*-B which is 0.0034% (v/v) ethanol resulted from initial concentration of 5% arabinose (w/v).

Keywords: Yeast, bioethanol, xylose, arabinose, *Durio zibethinus* L.**INTRODUCTION**

In the second generation of biofuel production, biomass has been attracting an attention for renewable energy development. Bioethanol produced as the alternative liquid fuel by fermentation using variety of substrates such as monosaccharides (xyloses and pentoses) and polysaccharides (starch). D-xylose and L-arabinose are pentose derived from hemicelluloses that have great potential as fermentation substrate (Yang, et al., 2015). To reduce competition using of edible sources or food as raw material is reason for us to utilize lignocellulosic biomass as raw material of biofuel especially for production of ethanol biofuel. Lignocellulosic biomass is abundant including empty fruit bunch of oil palm tree, sugarcane bagasse, rice straw, switchgrass, etc and it is considered as an attractive feedstock (Cardona, Quintero, and Pas, 2010; Chen, Wu, and Fukuda, 2008).

By converting lignocellulosic biomass from agricultural or household waste into bioethanol, it can overcome not only energy crisis problem, but also solves environmental problem. Lignocellulose is a complex biopolymer mainly composed of three

components: 35-50% cellulose, 20-32% hemicellulose, and 10-35% lignin (Sun and Sun, 2015). Glucose is the most abundant component in both cellulose and hemicellulose and can be fermented readily to produce ethanol. Pentose sugars in hemicellulose such as L-arabinose and D-xylose also could be converted into bioethanol (Olofsson, Bertsson, and Lidden, 2008). Thus, fermentation of all sugars content in the feedstock, hexose and pentose sugar should be considered in utilization of lignocellulose biomass (Bettiga, Bengtsson, Hahn-Hagerdal, & Gorwa-Grauslund, 2009).

One of the consideration for the successful fermentation determined by organism's ability to naturally ferment pentose sugars (arabinose and xylose) (Nidetzky, Novy, Krahulec, Lora, Klimacek, 2013). Hexose and pentose sugar which can be liberated by hydrolysis and fermented to ethanol. Generally, L-arabinose is part of pentose. It can be converted at high yields and rates together with the most abundant sugars, such as glucose and xylose (Maris, et al., 2010). Both xylose and arabinose need to be efficiently converted to consider the economic cost. They could be produced by some methods:

enzymatic hydrolysis, chemical synthesis, microbial fermentation, and acid hydrolysis (Zhu, Yu, Liu, Zh²⁶, and Zhang, 2017).

Saccharomyces cerevisiae has been utilized for producing ethanol in the beverage industry, and furthermore it has²² been utilized for producing bioethanol in recent decades (Hahn-Hagerdal, Galbe, Gorwa-Grauslund, Liden, and Zacchi, 20³¹). Wild type strain *S. cerevisiae* can't ferment either arabinose or xylose as carbon source to bioethanol. There are two ways to obtain microbial agent which can convert arabinose²⁵ xylose into bioethanol. Firstly, by developing a recombinant *S. cerevisiae* strain which can over-express of genes encoding enzymes in the pentose-phosphate pathway (Bettiga, et al., 2009) and secondly, screening for alternative microbes which could ferment xylose and arabinose as carbon source. In this study, we isolated some microbes from durian fruit (*Durio zibethinus* L.) which applied in fermentation either of arabinose or xylose as sole carbon source to produce ethanol as the innovation in developing bioethanol production. As we know that yeast cells are both saprophyte and parasite in fruit which contains simple sugars. Collonization of yeast is often indicated by synthesis of alcohol and will lead to the rotting of fruits (Ruriani, Sunarti, and Meryandini, 2012).

EXPERIMENTAL SECTION

Yeast strains and culture conditions

Yeast *Kodamaea ohmeri*, *Candida famata*, *Candida guilliermondii*, and *Cryptococcus laurentii* were isolated from durian fruit and identified by API 20C AUX Kit yeast identification system (BioMerieux). These yeasts were growing in² the agar media which are YPD medium contains 10g/L yeast extract, 20 g/L pe²tone, 20 g/L glucose; YPX medium contains 10g/L yeast extract, 20 g/L peptone, 20 g/L xylose; YPA agar medium contains 10g/L yeast extract, 20 g/L peptone, 20 g/L arabinose), agar media were prepared by adding and 20 g/l bacto agar into either YPD, YPX, or YPA media. These agar media contain MgSO₄·7H₂O, NaH₂PO₄, (NH₄)₂HPO₄, ethanol, NaOH, NaK tartrate.

Isolation of yeast from durian fruit (*Durio zibethinus* L.)

Four-yeast strains have been isolated from durian fruit (*Durio zibethinus* L.) which obtained from local market. Durian fruit in sterile water solution was prepared in various dilution 10⁻⁵ to 10⁻¹ by dissolved 10 gram of durian. The solution was spread on YPD agar medium containing 100µg tetracycline to inhibit bacterial growth, and incubated at 30 °C for 2 days. Single colonies were picked, purified, and further identified.

Yeast Identification

Yeast isolates were identified using yeast identification system API 20C AUX Kit (BioMerieux) following company's procedure with small

modification (Hermansyah, Adhiyanti, Julinar, Rahadiyanto, and Susilawati, 2017).

Phenotypic assay

Phenotypic assay was carried out according to previous procedure (Herma³ah, Novia, Sugiyama, and Harashima, 2015). Cell were grown on YPD, YPX, and YPA agar media and incubated at 30 °C for 2-4 day. Cell growth was observed whether sensitive or resistant to the media.

Fermentation test and analysis of ethanol content

The fermentation method used in this study according to described paper (Benjaphokee, et al., 2012) with some modification, especially to determine ethanol content using gas chromatography. Cell inoculum which grown in two days on YPD medium was transferred into YPD, YPA, and YPX media with nutrient. Nutrien⁶ for ethanol fermentation and analysis consisted of 1 g/l yeast extract, 0.5 g/L (NH₄)₂HPO₄, 0.025 g/l MgSO₄·7H₂O, and 0.1³⁰ NaH₂PO₄. Cultures were incubated with shaking at room temperature for 3 days to allow the ethanol establishment. Ethanol produced during fermentation were analyzed using gas chromatography (GC 2010 Shimadzu) under the following condition: column length and diameter 30 m, 0.25mm; N₂ carrier gas flow rate 136.3 mL/min, press²¹ 100 kPa; flame ionization detector, temperature of injector, column, and detector were 150 °C, 60-240 °C, and 200 °C, respectively. Ethanol production data were identified by comparing the initial arabinose and xylose substrate concentration with the ethanol yield that fermented by isolated yeast.

RESULTS AND DISCUSSION

Yeast isolates have been suc³⁵ fully isolated from durian fruit, and then identified using API 20C AUX kit (BioMerieux). This kit based on assimilation ass¹ where yeast isolates were grown on 19 sugars i.e. D-glucose, glycerol, calcium 2-keto gluconate, L-arabinose, D-xilose, adonitol, xylitol, D-galactose, inositol, D-sorbitol, metil-α-D-glucopyranoside, N-acetil-glucosamin, D-celliobiose, D-lactose, D-maltose, D-sucrose, D-trehalose, D-melezitose, D-rafinoase) comparing with control.

Based on the comparing result between observational and software database identification data from API 20C AUXkit (BioMerieux) as shown in Figure 1 and Table 1, six isolates (colony no 13, 14, 15, 16, 17, and 18) were identified as *K.ohmeri*, *C. famata* (two isolates designed as *C. famata*-A and *C. famata*-B), *C. guilliermondii*, and *C. laurentii* (two isolates designed as *C. laurentii*-A and *C. laurentii*-B). This result indicated that durian fruit contains various of yeast which may have a potent in some applications. It has also isolated and selected of suitable yeast strain f¹⁵ bioethanol fermentation in Brazil, and suggested the great biodiversity found in distillery environments could be an important source

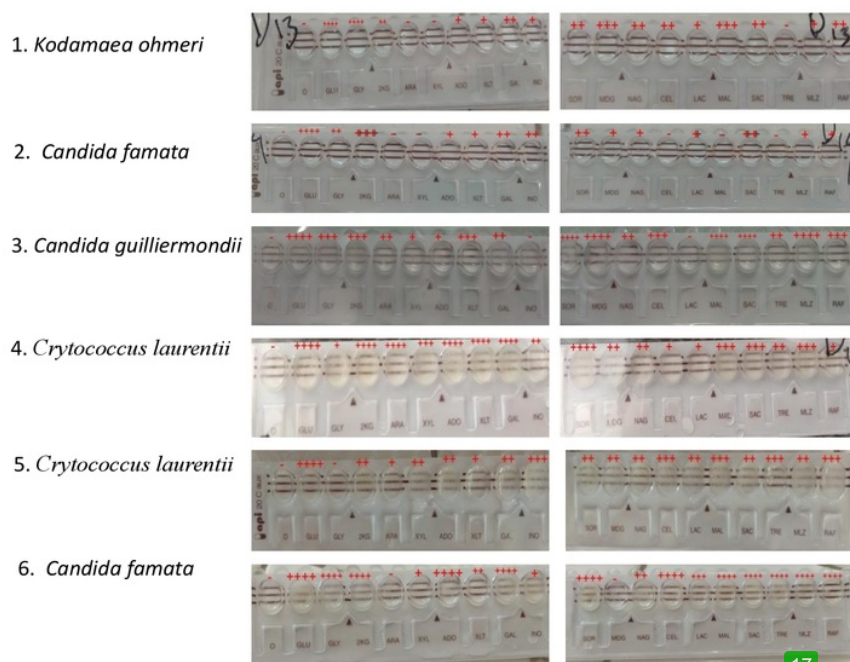


Figure 1. Identification 6 isolates using API 20C AUX kit (Biomerieux); 8 = negative control; Glu = D-glucose; Gly = glycerol; 2kg = calcium 2-keto gluconate; Mlz = D-melezitose; Mal = D-maltose; Sac = D-sucrose; Tre = D-trehalose; Nag = N-acetil-glucosamin; Cel = D = celliobiose; Raf = Rafinose; Ado = Adonitol; Xlt = xylitol; Gal = D-galactose; Lac = D-lacotose; Sor = D-sorbitol; Ino = inositol; Xyl = D-xylose; Ara = L-arabinose.

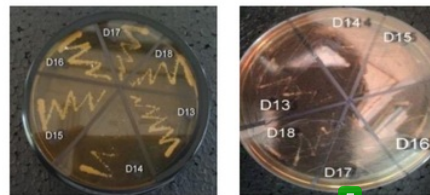
Table 1. Turbidity scale of isolates growth assay using API 20C AUX Kit

Sugar	D13	D14	D15	D16	D17	D18
7 (blank)	-	-	-	-	-	-
GLU	++++	++++	++++	++++	++++	++++
GLY	++++	++	+++	+	-	++++
2KG	++	+++	+++	++++	++	++++
ARA	-	-	++	++++	+	-
XYL	-	-	+	+++	++	+
ADO	+	+	+	++++	++	++++
XLT	+	+	+++	++++	+	++
GAL	++	++	++	++++	++	++++
INO	+	++	-	++	+++	+
SOR	++	++	++++	++++	++	++++
MDG	+++	+	++++	++	++	-
NAG	++	+	++	++	++	++
CEL	++	-	+++	+	+++	++++
LAC	+	+	-	+	++	+++
MAL	+++	-	++++	+++	+++	++++
SAC	++	++	++++	+++	++	++++
TRE	-	-	++	++	+++	++++
MLZ	+	+	++++	+++	++	++++
RAF	++	+	+++	+	+++	++++

Note : Turbidity scale = +++++: 100-76% ; ++++ : 75-51% ; +++ : 50-26% + : 0-25%



a) Isolates D13, D14, D15, D16, D17, and D18 on YPD media



b) Isolates D13, D14, D15, D16, D17, and D18 on YPA medium

c) Isolates D13, D14, D15, D16, D17, and D18 on YPX medium

Figure 2. Phenotypic test of yeast isolates D13, D14, D15, D16, D17, and D18 on YPD, YPA, and YPX media containing glucose (a), arabinose (b), and xylose (c) respectively as carbon source.

of strain (Basso, de Amorim, Oliveira, and Lopes, 2008).. However, they can't grow on all media test. Although cultures were cultivated for 5 days. They can only grow on media which contains specific sugar for growth. The ambient temperature favoured microbial growth incubated at 25 °C for 5 days (Voon, Hamid, Rusul, Osman, and Queck, 2006). Fatoni and Zufahair (2012) reported that tapioca waste hydrolyzed thermophilic amylase was fermented at temperature of 37 °C for 24 hours resulting 0.29% (v/v) ethanol. Phenotypic test was carried out to see characteristics of structural, biochemical, and physiological of isolates and this characteristic related to genotype and environment or their interaction. The results showed that yeast isolates grow faster and larger on YPD medium than on YPA or YPX media (Figure 2). This data indicated that yeast isolates utilized and assimilated glucose as carbon source better than arabinose or xylose.

Fermentation using microbial agent yeast isolates

To see whether yeast isolates can convert pentose sugars into ethanol, then these yeast isolates were applied as microbial agent to ferment arabinose and glucose as a sole carbon source in YPA, and YPX media. Fermentation of xylose and arabinose by microbial agent isolates *K. ohmeri* (D13), *C. famata* (D14 or *C. famata-A* and D18 or *C. famata-A*), *C. guilliermondii* (D15), and *C. laurentii* (D16 or *C. laurentii-A* and D17 or *C. laurentii-B*) was observed their ethanol product after 3 days incubating at room temperature with shaking. Shaking treatment does not allow a limited degree of headspace exchange and limit the evaporation, it will make the fermentation process more effective.

Based on gas chromatography data, it was found that the highest ethanol content was 0.021% (v/v) produced from 5% (w/vv) xylose substrate using *C. famata-A* (isolate D14) (Figure 3). As the goal of the fermentation is to find microorganism that effectively produce bioethanol from the second abundant sugar, xylose and arabinose. Furthermore, *C. Famata* work well at temperatures around 25 °C-30 °C and its natural habitat normally impose a continuous osmotic stress, due to low water activity in intracellular accumulation of polyhydroxy alcohol in order to fermented xylose. *C. famata* acknowledged as unicellular yeast, it is easier to distribute in a large-scale fermentor which is good reason for bioethanol production with higher yields (Sibiri, Fedorovich, Sybirna, Yatsyshyn, and Dmytruk, 2011). *C. famata* in legume-based fermented food and *C. Famata* preferentially colonize fruit surface in Japan (Hommel and Ahnert 1999).

While on the arabinose substrate, the highest ethanol content was 0.0034% (v/v) produced from 5% arabinone substrate using *C. laurentii-B* (isolate D17) (Figure 4). Generally plants contain abundant *C. laurentii* compared to other microorganism because it produce mycocins in soil. It is also one of fast growing yeast and appear after 24 hours of incubation at 30 °C (Grange, Rensburg, Ncube, and Malhuele, 2017). The various fruit, *Cryptococcus laurentii* known as antagonistic yeast that can remarkably induce resistance in pears, jujube fruits, peaches, table grapes, and cherry tomato and lessen postharvest fungal diseases in pear, apple, and strawberry (Tang, et al., 2019). Thus ability from *C. laurentii* might be become the reason why it presents in durian.

In this study, ethanol produced this fermentation was low concentration; we did not optimize the

condition. There are many factors that affected the condition such as pH, fermentation temperature, dissolved oxygen, and many others. Therefore, future experiment optimization should be conducted to obtain higher concentration. Xylose and arabinose has been fermented by recombinant *S. cerevisiae* with higher concentration of ethanol. The recombinant was constructed by inserting enzymes which involved in pentose utilization pathway. By the anaerobic condition, the recombinant strain fermented simultaneous co-utilization of xylose and arabinose

13 significantly reduced formation of the by-product xylitol, which contributed to improved ethanol production (Bettiga, et al., 2009). *S. cerevisiae* is important biocatalysis because its cost-effectiveness compared to the other fermenting agents that are produce zero chemical waste. Moreover, the production of bioethanol by *S. cerevisiae* has been taking key role in industry as an ideal biocatalysis for bioethanol production in sugar-containing nutrient medium (Monir, Aziz, Yousuf, and Alam, 2019).

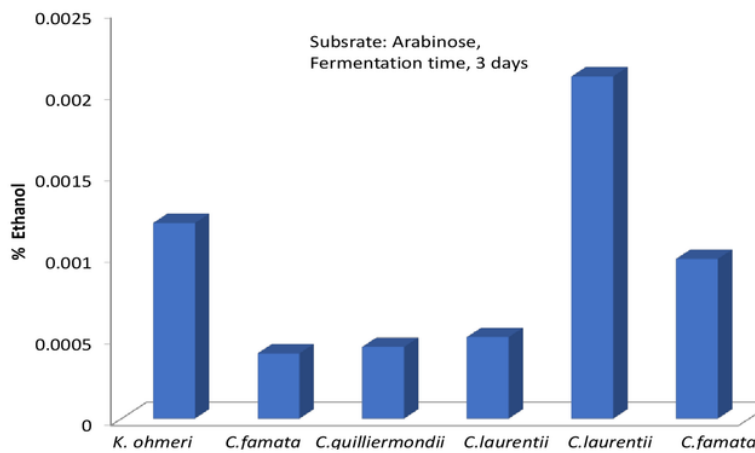
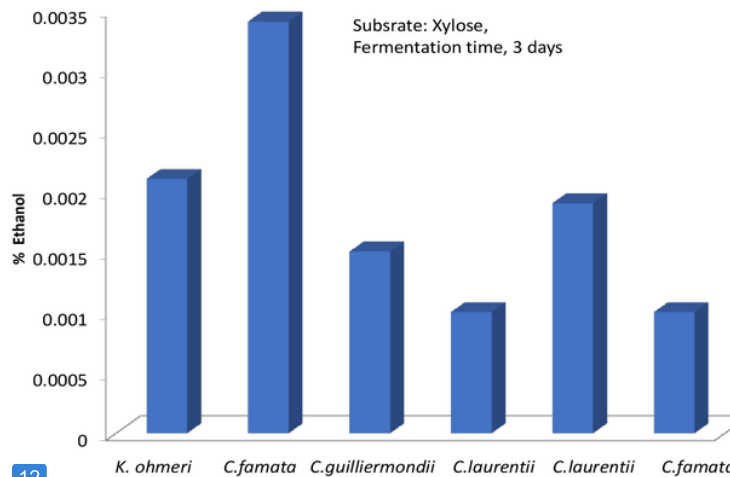


Figure 3. Fermentation of arabinose as sole carbon source using *K. ohmeri*, *C. famata* (two isolates designed as *C. famata*-A and *C. famata*-B), *C. guilliermondii*, and *C. laurentii* (two isolates designed as *C. laurentii*-A and *C. laurentii*-B) for three days.



12 Figure 4. Fermentation of xylose as sole carbon source using *K. ohmeri*, *C. famata*-A, *C. famata*-B, *C. guilliermondii*, and *C. laurentii*-A and *C. laurentii*-B for three days.

CONCLUSIONS

Six yeast isolates were identified from durian fruit, i.e. *Kodamaea ohmeri*, *Candida famata-A*, *Candida famata-B*, *Candida guilliermondii*, *Cryptococcus laurentii-A*, and *Cryptococcus laurentii-B*. Fermentation test showed that the highest ethanol content on 5% (w/v) xylose substrate achieved by *Candida famata-A* 0.021% (v/v) while 5% (w/v) of arabinose substrate fermented by *Cryptococcus laurentii-B* 0.0034% (v/v).

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