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Exploration Of Indigenous Yeasts As Inoculum In Fermentation Of Sugar Factory Waste (Bagasse) Into Xylitol Low-Calorie Sugar

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Abstract

Xylitol is a pentose sugar that has many benefits. The production of xylitol in biotechnology is more promising because in its production it only utilizes xylose fermentative yeast. This study aims to obtain yeast isolates and determine their ability to ferment xylose to xylitol. The samples used came from palm sap, coconut sap, soil and sugar press-mud. Yeast isolation was carried out by growing on YMA media which had added 0.1% chloramphenicol. Subsequently, the colony and cell morphology were observed. A total of 12 isolates of palm sap, 12 isolates of coconut sap, 8 isolates from soil and 6 isolates from sugar press-mud were screened on YPX Agar and Xylose Broth media with 3% xylose concentration to see their ability to grow on xylose media. Isolates P1, S3, KP3, KP4, T4 and B4 were selected for the fermentation test because they had the best growth in xylose broth as indicated by the highest OD value. Fermentation was carried out for 72 hours by measuring the pH value, total yeast at intervals of 24 hours. The xylitol levels formed were measured using HPLC/UPLC. Based on the measurement of the xylitol levels formed, the results were obtained in isolates P1 which was 1.28 g/100mL, S3 was 2.05 g/100mL, KP3 was 2.23 g/100mL, KP4 was 2.19 g/100mL, T4 2 was g /100mL and B4 2.47 g/100 mL with xylitol yields P1 which is 0.59 g/g, S3 is 0.67 g/g, KP3 is 0.63 g/g, KP4 is 0.62 g/g, T4 is 0.66 g/g and B4 were 0.64 g/g so that the six isolates isolated from palm sap, coconut sap, soil and sugar press-mud had the ability to produce xylitol.

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Keyword
Yeast, Xylitol , Sap, Soil, Sugar press-mud.

Introduction

Xylitol is a five-carbon sugar alcohol that has a sweet taste level similar to sucrose, but with lower calories, namely 2.4 Cal/g while sucrose is 4 Cal/g. Xylitol has many benefits such as as an alternative sugar for diabetics because it does not involve insulin in metabolic processes in the body.In addition, xylitol can also prevent otitis, osteoporosis, inflammatory processes and prevent carriers and improve oral health because xylitol can inhibit the growth of *Streptococcus mutans* bacteria that cause

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plaque on teeth (Sasaki *et al*. 2010; Kusumaningsari and Handajani 2011; Pal *et al*. 2013). So that currently the production of xylitol has been developed and has a high appeal.

Xylitol production is carried out by catalytic hydrogenation of pure D-xylose solution under high temperature and pressure. This process is very expensive and energy-intensive. One of the xylitol production strategies that are cheaper and more effective is biotechnology by utilizing microorganisms. Among the known xylose-using microorganisms, yeast is the best producer of xylitol and has been studied extensively compared to other microorganisms (Guo *et al*. 2006; Dasgupta *et al*. 2017). Yeast can convert xylose into xylitol because it has the enzyme Xylose Reductase (XR). Several types of yeast such as *Candida boidinii*, *Candida guilliermondii*, *Candida tropicalis*, *Candida magnolia*, *Debaryomyces hansenii*, and *Pichia assignitis* are known to have the ability to produce xylitol (Kumar *et al*. 2015).

Yeast can be found on various substrates such as fruits, seeds, animal skins, air, soil, water, and various substrates containing sugar (Suryaningsih *et al*., 2018). It is recorded in Pontoh (2012) that fresh sap contains sucrose 13.9-14.9%, ash content ranges from 0.22- 0.98%, protein ranges from 0.20-0.61% and fat content is 0.02 %. The nutritional content of sap provides suitable conditions for yeast growth.

In addition to sap, the presence of yeast in the soil is also very abundant, especially on sugar cane plantations. Sugarcane plantation soil is a suitable habitat for yeast growth because it is rich in organic matter derived from the decomposition of sugarcane litter which is also a source of nutrition for microorganisms (Botha, 2011).

On the other hand, the presence of sugar factory waste around sugar cane plantations and in the community is still common. Sugar factory waste can be in the form of solid, liquid, or gaseous waste. Solid waste in the form of sugar press mud, furnace ash, fly ash, and bagasse (bagasse). This waste can cause environmental pollution if not managed properly (Dharma *et al*. 2017; Fangohoy and Wandansari, 2017). Sugar press mud waste is one of the wastes that has not been utilized properly but still contains nutritional elements such as Na, K, Ca, phosphorus and sugar content of about 5-15% and still contains high organic matter, which is around 50%. The organic matter content can also increase the activity of microorganisms (Baig *et al*. 2002; Gupta *et al*. 2011; Juradi *et al*. 2020)

The presence of organic matter in sap, soil, and sugar press-mud can be a source of nutrition for yeast. Several studies reported the presence of yeast in soil, sap, and sugar press mud, namely those from the genera *Candida, Saccharomyces, Yorrowia, Brettanomyces, Endomycopsis. Rhodotula, Rotula* and *Debaryomyces.* Although various studies have shown the presence of yeasts in sap, soil, and sugar press mud, exploration of potential yeasts to produce xylitol is still rare and needs to be developed. Therefore, this study was conducted to obtain yeast from sap, sugarcane plantation soil, and sugar press mud waste which has the potential to ferment xylose to xylitol.

Materials and Methods

Materials

The sample of this study was yeast isolated from sap, soil, and sugar press mud. The sap used was palm sap and coconut sap obtained from one of the sap farmers in Pinrang Regency, while for sugarcane plantation land and sugar press mud waste from the Bone Sugar Factory in Bone Regency. Medium *Yeast Malt Agar* (0.3 g of yeast extract, 0.3 g of meat extract, 0.5 g of peptone, 1 g of glucose, and 2 g of agar for 100 mL). Medium *Yeast Peptone Xylose Agar* (1 g of yeast extract, 2 g of peptone, 3 g of xylose, 1.5 g of agar for 100 mL). *Medium Xylose Broth* (0.1 g of yeast extract and 3 g of xylose for 100 mL).

Yeast Isolation

A total of 1 g of the sample was diluted graded to 10-6. Then it was grown on *Yeast Malt Agar* (YMA) medium. After that, it was incubated in an incubator at 37 oC for 1x24 hours. After that, each colony showing a different morphology was regrown on the same medium to obtain pure isolates (Kanti and Latupapua, 2018).

Observation of Colony Morphology and Yeast Cell Morphology

The morphological oservations of yeast colonies were carried out using a stereo microscope with 40x magnification, to observe the characteristics of shape, color, margin, and elevation of yeast colonies growing on *Yeast Malt Agar* (Rahmana *et al*. 2016). While the morphological observations of yeast cell were carried out with methylen blue staining, to observe the characteristics of cell shape, budding, and the presence or absence of *pseudohyphae*. Observations using a binocular microscope with a magnification of 1000x (Rahmana *et al*. 2016; Citra, 2019).

Screening of Yeast to Grow on Media Containing Xylose

Preculture isolates were inoculated on Yeast Peptone Xylose Agar media with the quadrant scratch method to see the ability of yeast to grow on media containing xylose. The media was incubated at 37 oC for 48 hours. Isolate with the best growth on Yeast Peptone Xylose Agar media was then carried out a second screening using Xylose Broth media. Selected isolates from preculture were inoculated in sterile distilled water and diluted to obtain an OD value of 25% transmittance. A total of 1 mL was inoculated on 5 mL of Xylose Broth media and then incubated again for 24 hours at 37 oC. Cell growth was observed by measuring the optical density (OD) of each isolate using a spectrophotometer at a wavelength of 600 nm (Guo *et al*. 2006).

Xylose Fermentation

A total of 12 mL of inoculum that had been precultured and had the same OD value, was then inoculated on 120 mL of fermentation medium. The fermentation medium used were xylose 5.0 g, yeast extract 0.3 g, KH2PO4 0.5 g, MgSO4.7H2O 0.1 g, (NH4)2SO4 0.3 g for 100 mL. Then it was incubated for 72 hours at room temperature with a rotary shaker at a speed of 250 rpm (Yulianto *et al*. 2005).

Analysis of Fermentation Parameters

Total Yeast Calculation

Calculation of total yeast using the SPC (*Standard Plate Count*) method. A total of 1 mL of the fermented culture was put into 9 mL of sterile distilled water and carried out with graded dilutions. Furthermore, the last 3 dilution tubes were taken as much as 1 mL and inoculated with the pour plate method using *Yeast Malt Agar* (YMA) media then incubated at 37 oC for 24 hours. The total yeast calculation was carried out at 0, 24, 48, and 72 hours (Wiratno and Nofi, 2018)

Fermentation Culture pH Measurement

The measurement of the pH value was measured with a digital pH-meter. The pH meter was used by dipping the electrode into the sample to be measured. However, before using the tool, it was calibrated with a buffer of pH 7 and pH 4.

Xylose and Xylitol

Xylose content was measured using HPLC (High-Performance Liquid Chromatography) with 80 ± 5% Acetonitrile as mobile phase. Meanwhile, the measurement of xylitol levels was carried out using the UPLC (Ultra Performance Liquid Chromatography).

Data Analysis

The data from the isolation and observation of the morphology of the colonies and yeast cells were processed in the form of tables and figures and discussed descriptively. While the total yeast calculation data and pH measurements are displayed in graphical form. Meanwhile, the data obtained from the analysis of xylose and xylitol levels are presented in tabular form and discussed descriptively.

Results and Discussion

Yeast Isolations

The results of yeast isolation from palm sap, coconut sap, sugarcane plantation soil and blotong waste obtained 38 isolates. where 12 isolates were obtained from sugar palm sap, 12 isolates were obtained from coconut sap, 8 isolates were obtained from sugarcane plantation soil, and 6 isolates were obtained from Sugar press-mud. The determination of the selection of isolates was based on differences in the morphological characteristics of the colonies which included shape, color, elevation and margins of each of these yeast isolates.

Observation of Yeast Colony and Cell Morphology

The morphological observations of yeast colonies were carried out using a stereo microscope with a magnification of 40x. Colony morphology was observed directly from the purification results grown on YMA media. While the observation of yeast cell morphology using 0.1% methylene blue staining. The addition of methylene blue color to yeast cells was observed to show the difference between live and dead yeasts (Wachid & Mutia 2019).

Table 1. Colony and Cell Morphology

Isolate Source	Isolate code	Colony Morphology				Cell Mophology		
		Shape	Margin	Elevation	Color	Shape	Budding	*Pseudohyphae*
Palm Sap	P1	Round	Flat	Raised	White	Oval	Multipolar	Not Found
	P2	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	P3	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	P4	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	P5	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	P6	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	S1	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	S2	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	S3	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	S4	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	S5	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	S6	Round	Flat	Raised	White	Oval	Monopolar	Not Found
Coconut Sap	KP1	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	KP2	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	KP3	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	KP4	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	KP5	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	KP6	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	KS1	Round	Flat	Raised	White	Oval	Bipolar	Not Found
	KS2	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	KS3	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	KS4	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	KS5	Round	Flat	Raised	White	Oval	Monopolar	Not Found
	KS6	Round	Flat	Raised	White	Oval	Monopolar	Not Found
Soil	T1	Round	Filamentous	Raised	Creamy	Oval	Multipolar	Found
	T2	Round	Filamentous	Convex	Creamy	Oval	Multipolar	Found
	T3	Round	Filamentous	Convex	Creamy	Oval	Multipolar	Found
	T4	Round	Filamentous	Raised	White	Oval	Multipolar	Found
	T5	Round	Filamentous	Convex	White	Oval	Multipolar	Found
	T6	Round	Flat	Convex	Creamy	Oval	Monopolar	Not Found
	T7	Round	Flat	Convex	Creamy	Round	Monopolar	Not Found
	T8	Round	Flat	Convex	Creamy	Round	Monopolar	Not Found
Sugar Press mud	B1	Round	Flat	Convex	Creamy	Oval	Monopolar	Not Found
	B2	Round	Flat	Convex	Creamy	Oval	Multipolar	Not Found
	B3	Round	Flat	Convex	Creamy	Oval	Monopolar	Not Found
	B4	Round	Filamentous	Raised	Creamy	Oval	Multipolar	Not Found
	B5	Round	Flat	Convex	Creamy	Oval	Monopolar	Not Found
	B6	Round	Flat	Flat	Creamy	Oval	Monopolar	Not Found

Based on the results of observations of colony morphology on 12 yeast isolates obtained from palm sap, in general, they have the same shape, namely round, margins are flat, elevation is raised with the color of the colony, namely white, while for the results of cell morphology observations in general have the same cell shape, namely oval, with budding type that is monopolar except for isolates with code P1 with multipolar budding type, and for the presence of *pseudohyphae* of the 12 isolates not found.

The results of observations of colony morphology on 12 yeast isolates obtained from coconut sap in general have the same shape, namely round, margins that are flat, elevation that is raised with the color of the colony that is white, while for the results of cell morphology observations in general have the same cell shape, namely oval. , with budding type that is monopolar except for isolates with code KS1 with budding type that is bipolar, and for the presence of *pseudohyphae* of the 12 isolates not found.

The results of observations of colony morphology on 8 yeast isolates obtained from sugarcane plantation soils generally had the same shape, namely round, with colony margins that were filamentous for isolates T1, T2, T3, T4, and T5 and for isolates T6, T7, and T8, namely flat. The colony elevation was Convex for isolates T2, T3, T5, T6, T7, and T8, while for isolates T1 and T4 the elevation was Raised with the colony color being Cream for isolates T1, T2, T3, T6, T7, and T8 while for isolates T4 and T5 are white. As for the results of cell morphology observations in general, the cell shape is oval, except for isolates with codes T7 and T8 which are round with budding types, namely multipolar for isolates T1, T2, T3, T4, and T5, while for isolates with codes T6, T7 , and monopolar T8. For the presence of *pseudohyphae* for isolates T1, T2, T3, T4, and T5, *pseudohyphae* were found while for isolates with codes T6, T7, and T8 were not found.

The results of observations of colony morphology on 6 yeast isolates obtained from sugar pressmud generally had the same shape, namely round, with flat colony margins, except for isolate B4 which was Filamentous. The colony elevation was Convex for isolates B1, B2, B3 and B5, while for isolates B4 Raised and B6 Flat, the colony color in general was Cream. As for the results of cell morphology observations in general, the cell shape is oval, with the type of budding that is monopolar except for isolates B2 and B4 which are multipolar. For the presence of *pseudohyphae* from the 6 isolates, no *pseudohyphae* were found.

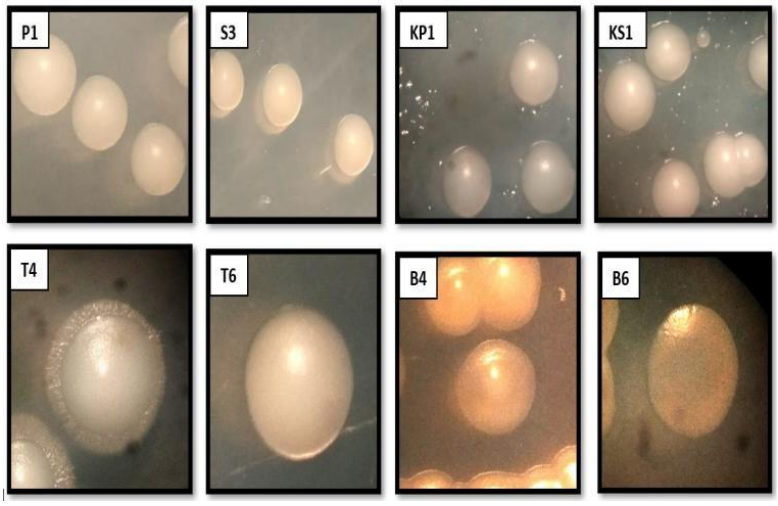

Figure1. Colony Morphology of Sap, Soil and Sugar press-mud

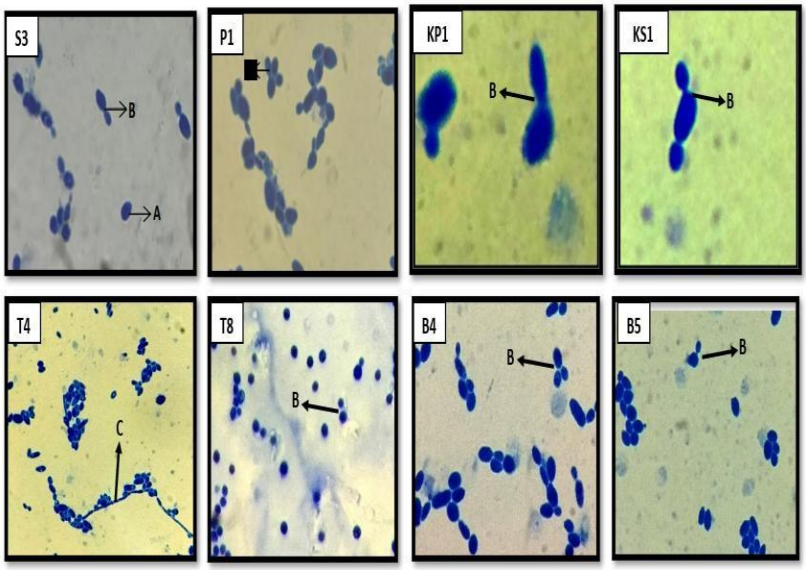

Figure2. Cell Morphology of Sap, Soil and Sugar Press-Mud; (A) Cell Shape; (B) Budding; (C) *Pseudohyphae*

Screening of Yeast to Grow on Media Containing Xylose

The screening process is the stage of selecting yeasts that can consume xylose as a carbon source. This stage was repeated twice by growing on agar and liquid media (broth). The first screening was carried out by growing 38 isolates purified on YPX Agar media using the quadrant scratch method and incubating for 2x24 hours. The concentration of xylose used in YPX Agar media is 3%. Screening on Agar media is intended to see the growth of yeast isolates on media containing xylose.

Based on the results of the first screening, it was found that from 38 isolates grown only 25 isolates were able to grow well on YPXA media, namely all isolates from palm sap, 6 isolates from coconut sap, namely KP1, KP2, KP3, KP4, KP5, KP6, 6 isolates from the soil, namely T1, T2, T3, T4, T5, T6 and one isolate from sugar press mud, namely B4. Twenty- five isolates can consume xylose as a carbon source. Xylose is a pentose sugar that not all yeasts can consume, it is influenced by the presence of the enzyme Xylose Reductase (XR) where the enzyme will be activated when there is xylose (Muller,2009).

After being grown in YPX Agar media, the 25 isolates were then continued for a second screening by growing them in xylose broth media with 3% xylose media composition. Before being put into the xylose broth medium, the isolate was first diluted to reach a transmittance value of 25% to equalize the cell biomass entering the xylose broth medium. Then incubated for 1x24 hours. After incubation, the Optical Density (OD) value of each isolate was measured using a spectrophotometer with a wavelength of 600 nm. The measurement of the OD value was carried out to see the level of turbidity of the isolated culture media where the higher the OD value, the higher the amount of cell biomass in the culture medium.

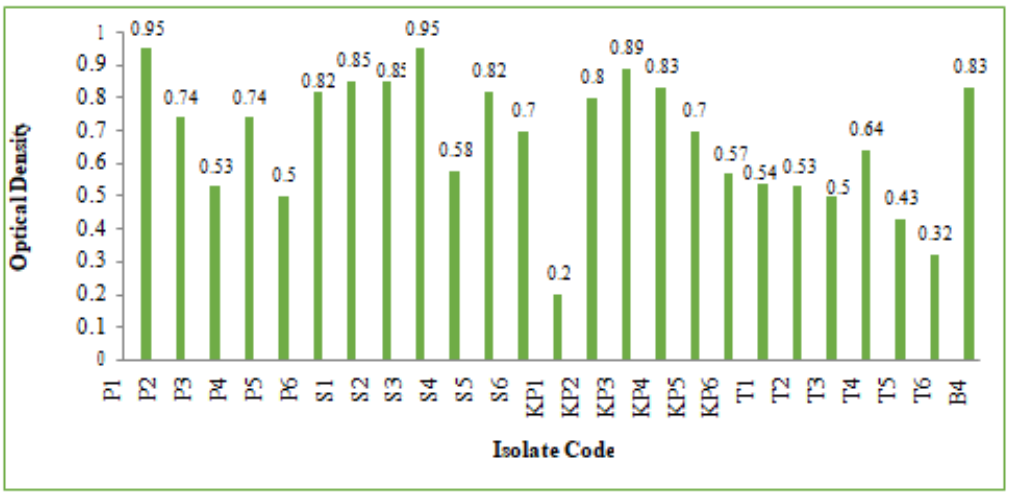

Figure 3.Graph of OD values

The results of the measurement of OD values are presented in (Figure 3) of 25 isolates grown on xylose Broth media, 6 isolates were selected from 4 different isolate sources which showed the highest optical density (OD) value, namely 2 isolates from palm sap (P1 and S3) with an OD value of 0.95, 2 isolates derived from coconut sap, namely KP3 with an OD value of 0.89 and KP4 with an OD value of 0.83. B4 from soil with an OD value of 0.83, and T4 from blotong with an OD value of 0.64. The difference in OD values was due to differences in the ability of yeast to use xylose within 24 hours, thus affecting the growth and number of yeast cells in liquid media. Mardawati *et al*. (2018) explained that each yeast has a different metabolic rate in converting xylose to xylitol.

Fermentation Xylose to Xylitol

The fermentation test is intended to see the ability of yeast to ferment xylose to xylitol within 72 hours. Before the fermentation test, the six selected isolates were first grown in preculture media for 1x24 hours to adjust the growth of yeast before entering the fermentation medium. Fermentation was carried out by growing isolates P1, S3, KP3, KP4, B4, and T4 which had been precultured into fermentation media with 5% xylose concentration. In addition, a fermentation medium was made without adding yeast isolate as a control (comparison) in the fermentation test. The concentration of cells entering the fermentation medium was equated with an OD value of 0.6 or 25% Transmittance using a wavelength of 600 nm.

During fermentation, total yeast was calculated using the *Standard Plate Count* (SPC) method and measured pH values every 0, 24, 48, and 72 hours or 24-hour intervals for 3 days. The results of fermentation for 72 hours have then measured the levels of xylitol formed and the levels of xylose used using HPLC/UPLC

Total Yeast

From the results of the total yeast calculation, the growth graph is obtained as follows :

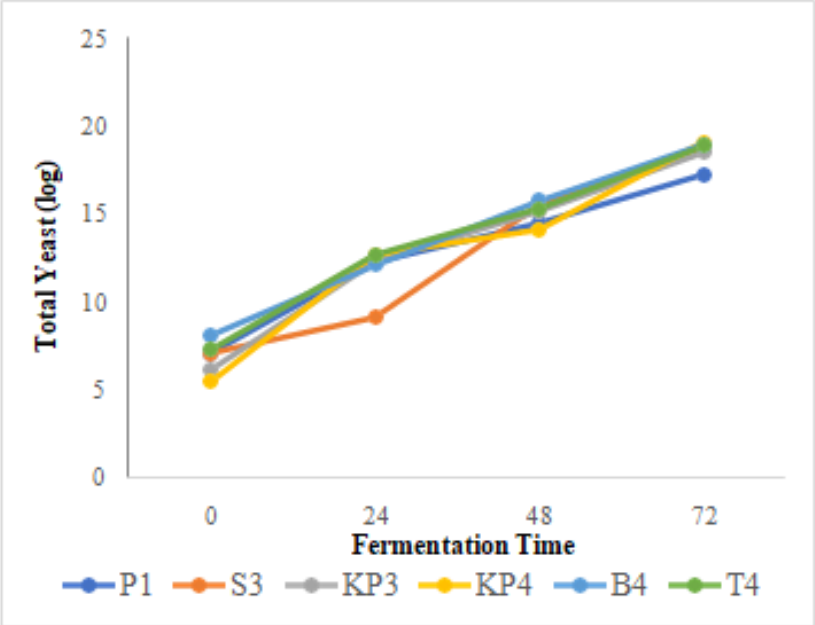

Figure4. graph of Total Yest

Based on the graph of total yeast growth, it was known that yeast cells increased steadily with increasing fermentation time. The amount of yeast at the beginning of the fermentation continued to increase until the 72nd hour. where isolates P1 and S3 with an initial yeast count of 1.2 x 10^{10} CFU/mL increased in isolates P1 of 1.8 x 10^{19} CFU/mL, S3 by 6.8 x 10^{20} CFU/mL. For isolates, KP3 showed an increase from the initial number of the incubation period of 1.3 x 10^{8} CFU and increased to a total of 3.24 x 10^{20} CFU/mL and KP4 with an initial amount of yeast of 2.7 x 10^{8} CFU/mL continued to increase up to 1.3 x 10^{21} CFU/mL. of 9.4 x 10^{20} CFU/mL. While isolate B4 from the initial yeast count, namely 1.2 x 10^{10} CFU/mL, continued to increase to 9.4 x 10^{20} CFU/mL and T4 also showed the same increase in total yeast, where the initial yeast total was 1.8 x 10^{9} CFU/mL to 8.8 x 10^{20} CFU/mL. As for the control, cell biomass was not added so that it did not show cell growth.

Based on the graph of the total yeast count (Figure 4), it can be seen that the yeast cells of the six isolates were still in the growth phase. The increase in total yeast indicates that during the fermentation process the yeast cells continue to grow. With the increase in the number of cells, metabolic activity increases (Usmiati and Marwati, 2007).

Based on the graph of the total yeast count (Figure 4), it can be seen that the yeast cells of the six isolates were still in the growth phase. The increase in total yeast indicates that during the fermentation process the yeast cells continue to grow. With the increase in the number of cells, metabolic activity increases (Usmiati and Marwati, 2007).

Measurement of pH

Measurement of pH was carried out to determine changes in pH during the fermentation process. The following are the results of measuring the pH value of each fermentation inoculum:

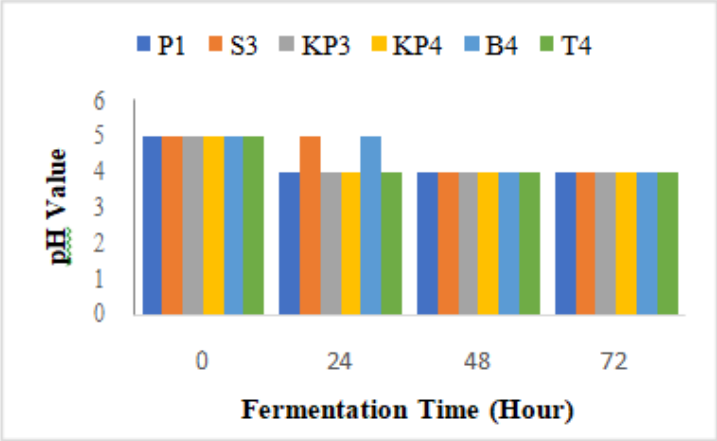

Figure 5. Graph of pH

Based on the results of pH measurements, it is known that the initial pH of all fermentation media after adding inoculum is pH 5. According to Fairus *et al*. (2013) a good pH value for the growth of yeast Candida sp. is a xylose user yeast with a pH range of 4-6. All isolates experienced a decrease in pH to 4 after 72 hours of fermentation. The decrease in pH is caused during the fermentation process which tends to form organic acids which can lower the pH. According to Yulianto (2001), during the xylose fermentation process, acetic acid will be formed which can lower the pH. In this study, the optimum pH for the tested yeast is not yet known. However, it is known that the pH of each fermentation medium has met a good pH range for fermenting xylose.

Analysis of Fermentation Yield

Analysis of xylose and xylitol levels in the six isolates and controls was carried out after 72 hours of the fermentation process which was measured using HPLC (High-Performance Liquid Chromatography) and UPLC (Ultra Performance Liquid Chromatography). Measurement of final xylose and xylitol levels is intended to see the levels of xylitol produced by yeast by utilizing used xylose within 72 hours.

The initial xylose concentration used in the fermentation was 5 g/100mL. The results of xylitol production by yeast isolates are presented in (Table 6), which shows that the results of measuring xylose levels obtained final xylose at P1 of 2.85 g/100mL, isolate S3 of 1.94 g/100mL, KP3 of 1.50 g /100mL, KP4 is 1.52 g/100mL, B4 is 1.15 g/100mL and at T4 is

1.97 g/100mL. So it can be seen that the substrate or xylose used in the fermentation has not been consumed by the yeast to produce xylitol during the 72 hour fermentation time.

Table2. Analysisof Fermentations Yield

	Initial Xylose Concentration (g/100mL)	Final Xylose Concentration (g/100mL)	Xylose Used (g/100mL)	Final Xylitol Concentration (g/100mL)	*Yield* Xylitol ($Y_{p/s}$) (g/g)
P1	5	2,85	2,15	1,28	0,59
S3	5	1,94	3,06	2,05	0,67
KP3	5	1,50	3,50	2,23	0,63
KP4	5	1,52	3,48	2,19	0,62
B_4	5	1.15	3.85	2.47	0.64
T_4	5	1.97	3.03	2	0.66
Control	5	5	-	-	-

This is in line with the yeast growth graph (Figure 4) which still shows the growth phase so that the consumed xylose has not been used up. The final xylose content in the control was still 5 g/100mL and no xylitol production was found.

The results of the measurement of the final xylitol level obtained in S3 are 2.05 g/100mL, P1 is 1.28 g/100mL, KP3 is 2.23 g/100 mL, KP4 is 2.19 g/100 mL, B4 is 2.47 g/100mL and T4 is 2 g/100mL. Based on these results, it is known that the isolates that produced the highest xylitol levels were isolated B4 with xylitol levels of 2.47 g/100mL.

The results of the calculation of xylitol production in yeast are expressed in xylitol product yield (Yp/s) (g/g). According to Safitri *et al*. (2016) product yield (Yp/s) is the number of nutrients or substrate (xylose) used by yeast to form products (xylitol). The xylitol yield obtained in isolates P1 was 0.59 g/g, S3 was 0.67 g/g, KP3 was 0.63 g/g, KP4 was 0.62 g/g, B4 was 0.64 g/g while in T4 is 0.66 g/g. The yield obtained in isolate P1 was higher than that in isolate B4, meaning that the efficiency of using xylose in P1 to form xylitol was higher than that in isolate B4 although the final xylitol yield obtained in B4 was higher.

According to Barbosa *et al*. (1988) in Arifan and Nuswantari (2020), stated that the theoretical maximum value of xylitol production yield is 0.917 g/g. Based on the xylitol yield value, it was still below the maximum yield of xylose fermentation. This can be influenced by factors that support the production of xylitol. Azizah (2019), explained that several factors influence the fermentation process to produce xylitol, namely carbon and nitrogen sources, temperature, substrate concentration, and aeration. In addition, during the fermentation process, it allows the formation of inhibitor compounds such as acetic acid which can reduce xylitol production. Silva *et al*. (2004) explained that acetic acid can affect yeast cell metabolism in producing xylitol where acetic acid will reduce yeast cell growth. Thus, this study found that isolates isolated from palm sap, coconut sap, soil, and sugar press mud had the potential to ferment xylose to xylitol for 72 hours of fermentation.

Conclusions

Based on this research it can be concluded that:

1. The results of yeast isolation from sap were 24 isolates, consisting of 12 isolates from sugar palm sap samples and 12 isolates from coconut sap samples, 8 isolates from the soil, and 6 isolates from sugar press mud where only 25 isolates were able to ferment

xylose, namely 12 isolates. palm sap, 6 isolates from coconut sap, 6 isolates from the soil, and 1 isolate from the soil.

2. Six yeast isolates from sap, soil and sugar press mud, namely isolates P1, S3, KP3, KP4, T4 and B4 were able to ferment xylose to xylitol with final xylitol levels respectively 2.05 g/100mL, 1.28 g/100mL, 2.23 g/ 100 mL, 2.19 g/100 mL, 2.47 g/100mL and 2 g/100mL with final xylitol yields of 0.59 g/g, 0.67 g/g, 0.63 g/g, 0.62 g/g, 0.64 g/g, respectively. and 0.66 g/g with a fermentation time of 72 hours.

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