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Original Article

Potential of xylose-fermented yeast isolated from sugarcane bagasse waste for xylitol production using hydrolysate as carbon source

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Abstract

Xylitol is a high value sugar alcohol that is used as a sweetener. In the past years, the biological process of D-xylose from lignocellulosic material into xylitol has gained increasing interest as an alternative production method. In this study, sugarcane bagasse was used as raw material for xylitol production because of its high efficiency, reduced industrial cost, and high concentration of xylose. Pre-treatment of sugarcane bagasse with sulfuric acid was performed with various conditions. The results showed that the optimum condition was exhibited for 3.1% sulfuric acid at 126°C for 18 min producing 19 g/l xylose. Isolated yeasts from the sugarcane bagasse were selected and tested for xylitol ability from xylose. Results showed that Candida tropicalis KS 10-3 (from 72 isolates) had the highest ability and produced 0.47 g xylitol/ g xylose in 96 hrs of cultivation containing 32.30 g/l xylose was used as the production medium.

Keywords: acid hydrolysate, sugarcane bagasse composition, xylose-fermenting yeast, xylitol

1. Introduction

Xylitol ($C_5H_{12}O_5$) is a natural five-carbon sugar alcohol; recently it has become very interesting because of its use as an industrial food sweetener which can be used by diabetics' patients, as it enters the bloodstream slowly and is a natural insulin stabilizer. Xylitol is anticacinogenic and prevents the formation of acids that attack the tooth enamel. Moreover, xylitol has the ability to inhibit the growth of oral bacterial species, which cause acute otitis medium including *Streptococcus pneumonia* and *Haemophilus influenza*, (Rao *et al*., 2006; Jeevan *et al*., 2011; Rubio *et al*., 2012). Xylitol is found in various fruits and vegetables, but some are not used for xylitol extraction because their low content, less than 9 mg/g, makes manufacturing expensive (Rubio *et al*., 2012). Xylitol is currently produced on an industrial scale by a catalytic reduction (hydrogenation) of xylose obtained from wood sources, such as white birches. There are some disadvantages because a xylose purification step is necessary and makes the process expensive. The hydrogenated solution produced requires further processing (chromatographic fractionation, concentration and crystallization) to attain pure xylitol. About 50-60% of the xylose is converted into xylitol, and the refining and separation steps are more effective. Xylose obtained from hemicellulose hydrolysates can be used for xylitol production by hydrogenation of xylose sugar at 80-140°C and hydrogen pressures of up to 50 atm in the presence of Raney nickel catalyst (Paraj *et al*., 1998). However, the biotechnological process based on the utilization of various microorganisms, such as bacteria, mold, and yeasts

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that can convert xylose into xylitol, a highly specific and economic process since 80% of the sugar is transformed into sugar alcohol. The biotechnological method uses mostly potential agricultural waste as substrate which is low cost and environmental friendly (Rubio *et al*., 2012). Microbial xylitol production from agricultural wastes containing hemicelluloses could be suitable because this effectively uses renewable resources. In general, among xylose-producing microorganisms, the best xylitol producers are yeasts especially genus *Candida* (Fabio *et al*., 2008) such as *Candida parapsilosis,C. guilliermondii, C. intermedia, C. boidinii*, *C. mogii, C. shehatae, C. tenuis, C. tropicalis, C. utilis, Debarvomvces hansenii, Hansenula anomala, Kluvveromvces franilis, K. marxianus, Pachysolen tannophilus, Pichia stipites* and *Schizosaccharomyces pombe* (Winkelhuazen and Kuzmanova, 1998; Fabio *et al*., 2008; Ghindea, 2010).

Sugarcane bagasse, *Saccharum officinarum* L., is a byproduct of the extraction process in sugar production. For each mill the generated bagasse makes 35-40% of the weight of the milled sugarcane. Bagasse contains mostly lignocelluloses composed of lignin, cellulose, and hemicellulose. Lignocellulose from sugarcane bagasse is a substrate which is available in abundance, widespread, cost effective, and an economical source of biomass. There are many sources of sugarcane bagasse all over MahaSarakham Province in Thailand because of a high number of sugarcane factories for processing sugarcane cultivation. Sugarcane bagasse is composed of hemicellulose, a good resource for producing D-xylitol, D-glucose, D-galactose, D-mannose, D-xylose, D-arabinose and D-glucuronic acid with acetyl side chains (Chen *et al*., 2010). The objective of this research was to isolate xylose fermenting-yeast from sugarcane bagasse waste and evaluate the xylitol production using the bagasse acid hydrolysate as a carbon source.

2. Materials and Methods

2.1 Preparation of sugarcane bagasses hydrolysate

The sugarcane bagasse was pretreated with different concentrations of sulfuric acid with a sugarcane bagasse and sulfuric acid ratio of 1:10, hydrolysis including 1%, 2%, and 3.1% v/v to determine the highest yield of xylose. After pre-treatment the sugarcane bagasses were hydrolyzed under different temperatures based on the acid concentration by 1% v/v) H_2SO_4 concentration 121°C, 60 min, Rao *et al*., 2006), 2% v/v) H_2SO_4 134°C, 60 min, Jeon *et al*., 2010) and 3.1% v/v) H_2SO_4 126°C, 18 min, modified of Paiva *et al*., 2008) in an autoclave. The liquid fraction was then filtered through Whatman no.1 filter paper and the pH was raised to 9 with calcium oxide and then decreased to 5.5 using phosphoric acid. After this, the hydrolysate was mixed with activated charcoal concentration 1, 2.5 and 3% w/v, and agitated 150 rpm, 30°C, 60 min. The hydrolysate was then concentrated under vacuum at 70°C to increase the xylose concentration using an evaporator (Buchi Rotavapor® 215+v-700/ v-855). The sugarcane bagasses hydrolysate was used as a fermentation medium for xylitol production.

2.2 Isolation of xylose fermenting-yeasts and culture conditions

Samples of sugarcane bagasse waste were obtained from sugar factories in MahaSarakham and Khon Kaen Province, Thailand. For screening, 10 g of each sample was placed into 90 ml of malt extract-xylose-yeast extract-peptone, MXYP, medium containing 30 g/l xylose, 3 g/l yeast extract, 3 g/l malt extract, and 5 g/l peptone, in a 250 ml Erlenmeyer flask and incubated at 30°C for 72 hrs The enriched cells were spread on MXYP agar plates, 100 g/l xylose. After incubation at 30°C for 48 hrs, the cross streak method was used to obtain pure isolates individual colonies of microorganism. The isolated yeast were picked up and maintained on slant YM agar (containing 4 g/l yeast extract; 10 g/l malt extract, 4 g/l glucose and 20 g/l agar) at 30°C for 48 hrs, maintained at 4°C and subcultured at regular intervals. The assays were carried out in MXYP medium, containing 10 ml MXYP medium (initial xylose 30 g/l) and cultivated on a shaker incubator at 150 rpm/ min and 30°C. Cell growth was measured by optical density at 600 nm. After 48 hrs cultivation, samples of each strain were analyzed.

2.3 Inoculum development

Six isolated xylose fermented-yeasts from xylose assimilation tests including *C. guilliermondii* from the Thailand Institute of Scientific and Technological Research, TISTR, were used for xylitol production. From the subculture, one loopful of yeast cells was inoculated into 100 ml Erlenmeyer flasks containing 50 ml of MXYP medium, and then cultivated at 30°C for 24 hrs on a rotary shaker at 150 rpm. Inoculated cells were counted using a haemacytomer; final cell concentration was $1x10^8$ cells/ ml and used as an inoculum in xylitol fermentation process.

2.4 Fermentation

Flask batch fermentations were performed by shaking in 125 ml Erlenmeyer flasks containing 50 ml of sugarcane bagasses hydrolysate (initial xylose concentration 32.30 g/l) into a fermentation medium, containing 2 g/l $(NH_4)_2SO_4$, 0.5 g/l $MgSO_4.7H_2O$, 10 g/l yeast extract, 20 g/l peptone, 0.5 g/l KH_2PO_4, and 0.5 g/l K_2HPO_4, and cultivated in a rotary-shaker at 150 rpm, 30°C for 96 hrs. They were inoculated to a final concentration of 10^8 cells/ ml. The samples were collected at regular intervals of 24, 48, 72, and 96 h. of incubation. Aliquots of the cultures were centrifuged at 12,000 xg for 10 min and the supernatant subjected to high performance liquid chromatography (HPLC) analysis for determining the sugar consumption and xylitol concentration.

2.5 Strain identification

Partial sequences of the D1/D2 domain approximately 600-800 bp of the LSU rDNA and the SSU rDNA were determined from PCR products from genomic DNA extracted from yeast cells by using a slightly modified version of the method (Lachance *et al*., 2001). A divergent 5' domain of the gene was amplified by a PCR with the forward primer NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG) and the reverse primer NL-4 (5'-GGTCCGTGTTTCAAGACGG) (O'Donnell, 199); amplification of the SSU rDNA was done with the forward primer P1 and the reverse primer P2 (Sjamsuridzal *et al*., 1997). DNA product was subjected to electrophoresis on 3% agarose gel, recovered using the QIAquick purification kit (Qiagen) and cycle-sequenced using the ABI Big Dye terminator cycle sequencing kit, version 3.1 (Applied Biosystems), with the external primers NL-1 and NL-4 for the D1/D2 domain (Kurtzman and Robnett, 1998) and eight primers, P1–P8, for the SSU rDNA (Yamada *et al*., 1999). The sequences were determined with an ABI PRISM 3100 automated DNA sequencer (Applied Biosystems) according to the instructions of the manufacturer. Sequences data base was compared with the BLASTN homology search (http://www.ncbi.nlm.nih.gov/BLAST/, NCBI) and generated sequences were aligned with related species retrieved from GenBank using the CLUSTAL X, version 1.81 (Thompson *et al*., 1994).

2.6 Analytical methods

Xylose, glucose, arabinose, acetic acid, HMF, furfural, phenolic compound and Xylitol concentrations were determined using HPLC with an Aminex HPX-87H, (Biorad, USA) carbohydrate column (300 x 7.8 mm) at 45°C, using 5 mM H_2SO_4, as an eluent. A flow rate of 0.5 ml/min and a sample volume of 20 µl were maintained. The eluate was monitored with refractive index (RI) detector. Peaks were identified and quantified by comparison with the retention times of authentic standards (Xylose and Xylitol purchased from Sigma Company).

3. Results and Discussion

3.1 Sugarcane bagasses hydrolysis

Sugarcane bagasse is a lignocellulosic material waste from sugar mills consisting of cellulose, hemicellulose, lignin and other compounds particularly various types of sugar containing five and six carbon atoms it is commercially used as a carbon source to produce a variety of products. The sugars produced from sugarcane bagasse are glucose, galactose, mannose, xylose, and arabinose. Galacturonic acid can be produced from dilute acid or base hydrolysis. For the remaining sugars in the bagasse there is a breakdown of the beta-glucosidic bond that has a monosaccharide. Chandel *et al*. (2011) illustrate that auto hydrolysis is a simple method to break down the hemicellulosic backbone into monomeric sugar constituents with fewer by-products. However, a significant fraction of hemicellulosic may remain with the substrate after the auto hydrolysis. To overcome this problem, dilute acid hydrolysis is used for maximum degradation of hemicelluloses into monomers. Compared to other pretreatment methods, dilute acid hydrolysis is more useful for the conversion of maximum hemicellulosic fraction into xylose and other sugars, which can be fermented by specialized microorganisms.

The results showed that the maximum xylose concentration of 19.0 g/l was obtained at 3.1% sulfuric acid concentration (126°C, 18 min) (Table 1). The increase in sulfuric acid concentration enhanced the decomposition of lignin and the xylose sugars released from the sugarcane bagasses during acid hydrolysis more other methods. Lignin was broken down into phenolic compounds, such as vanillin, syringaldehyde, 4-hydroxybenzoic acid and ferulic acid which have a toxic effect on microbial growth and xylose metabolism in yeast cells.

According to recent research on the use of dilute sulfuric acid to digest sugarcane bagasse Rao *et al.* (2006) found that using sulfuric acid concentration of 1% v/v under a temperature of 121°C, 60 min produced xylose, glucose and arabinose at 56, 15, and 24%, respectively. Paiva *et al.* (2009) have reported that sulfuric acid concentrations of 3.1% v/v at a temperature of 126°C, 18 min gave xylose at 266.73 mg/g dry weight of sugarcane bagasse. Jeon *et al*. (2010) used sulfuric acid concentrations of 2% v/v under a temperature of 134°C, 60 min. and produced xylose, glucose and arabinose at 21, 5.4, and 3 g/l, respectively. They used dilute acid as a catalyst in the hydrolysis. Hemicellulose and lignin dissolved mostly at low concentrations (0.05-5%) this will minimize damage to products and offer the highest sugar yield at the end of the process. Furthermore, dilute acid enhances the economic potential for industrial production, since it is easy to control and can treat large amounts of the biomass in a short time. The mechanism of the reaction of acid hydrolysis are as follows, (1) diffusion of protons through the matrix of lignocellulosic wet, (2) heterocyclic cyclic bonding of oxygen protons and sugar monomers, (3) the ether bond is broken, (4) intermediate carbocation, (5) the solubility of carbocation with water, (6) the restoration of protons with the sugar energy monomer, (7) the distribution of the reaction in the liquid phase, and (8) the resumption of the second stage, which is developed from the experiments model (Aguilar *et al*., 2002).

Results in Table 2 show that toxic compounds are byproducts obtained from the acid hydrolysis step and they could be removed by charcoal. A main disadvantage of the synthesis of lignocellulosic, which comes from the diluted acid hydrolysis process, is the degradation of the many sugars type in hydrolysis processes and the formation of undesirable by-products, which inhibit the fermentation process. The by-products in diluted acid concentrations are divided into three main groups: (1) weak acids, e.g. acetic

Table 1. Sugar composition of sugarcane bagasse pre-treatment by different conditions.

Condition	Sugar composition (g/l)		
	Xylose	Glucose	Arabinose
H_2SO_4 1%Temp. 121°C, 60 min.	18.51	2.42	2.20
H_2SO_4 2%Temp. 134°C, 60 min.	12.06	3.23	2.17
H_2SO_4 3.1%Temp. 126°C, 18 min.	19.00	2.75	2.63

Table 2. Sugarcane bagasse hydrolysate composition.

Treatment	Sugar composition (g/l)			Toxic compound (g/l)			
	Xylose	Glucose	Arabinose	HMF	Furfural	Acetic acid	Phenolic compound
Original	14.95	1.27	3.13	0.02	0.03	3.90	5.24
Evaporation	44.58	5.50	8.00	0.07	0.09	5.54	7.78
H + Charcoal 1%	32.13	5.98	7.59	0.03	0.05	2.37	0.25
H + Charcoal 2.4%	31.62	5.78	7.54	0.03	0.04	1.85	0.19
H + Charcoal 3%	32.30	5.90	7.60	0.02	0.04	1.56	0.15

H = Hydrolysate

and formic acids, (2) furan derivatives, e.g. furfural and 5-hydroxymethylfurfural, and (3) phenolic compounds. Several detoxification methods have been reported to overcome the inhibitory effect of these compounds during fermentation by yeasts, such as adaptation of microorganisms to the medium, treatments with molecular sieves, ion-exchange resins or charcoal, steam stripping and overtitration to remove various inhibitory compounds from lignocellulosic hydrolysates, especially charcoal cane effective remove some toxin compounds owing to the toxic substance attaches to the surface of the charcoal. Kamal *et al*. (2011) and Aguilar *et al*. (2002) presented activated charcoal that has been widely used in the removal of carbon compounds hydrolysate concentration; contact time greatly influenced the removal of toxicity compounds. Different proportions varying from 1% to 30% in addition, they observed that 1% of charcoal was enough to remove 94% of the phenolic compounds. Mussatto and Roberto (2001) reported an increase in xylitol production by *Candida guilliermondii* using cells in the past appropriate to hemicellulosic hydrolysate of rice straw. This work, detoxification using activated charcoal concentration 3% (w/v) benefits the xylitol production by removing inhibitors because the toxic substance attaches to the surface of the charcoal and maximizes xylose residual sugar from the hydrolysate.

3.2 Isolation of xylose fermented-yeasts and culture conditions

Isolation xylose fermenting-yeasts have the ability to use xylose as a carbon source. Samples of sugarcane bagasse waste, collected from sugar factories in Kosum Phisai, MahaSarakham Province and Phu Wiang, Khon Kaen Province, led to the isolation of yeasts, 34 and 38 isolates, respectively. Morphological characteristics shown in Table 3 demonstrate xylose-fermenting yeast of sugarcane bagasse waste, from sugar factories, at Kosum Phisai, MahaSarakham Province, 34 isolates exhibit 25 isolates of white colony, 4 isolates of cream colony, 4 isolates of pink colony and 1 isolate of yellow colony. From Phu Wiang, Khon Kaen Province, 38 isolates presented 25 isolates of white colony, 11 isolates of cream colony, 1 isolate of pink colony and 1 isolate of yellow colony.

The sugar xylose contains five-carbon atoms and is converted into xylitol by microbial fermentation using bacteria, fungi and yeast. The best known xylitol producers are yeasts, with special emphasis on the genus *Candida* (Ikeuchi *et al*., 1999; Kang *et al*., 2005; Guo *et al*., 2006; Sampaio *et al*., 2008) and *Debaryomyces* (Altamirano *et al*., 2000; Sampaio *et al*., 2008; Prakash *et al*., 2011). Microbial

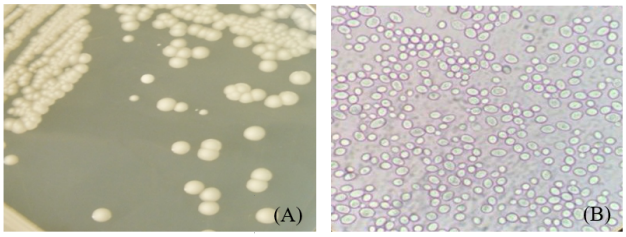

Figure 1. Characteristics of xylose fermented-yeast strain KS 10-3. (A) Colony of KS 10-3 strain on YM agar plate. (B) KS 10-3 from bright microscope (40X).

Table 3. Morphology and growth ability in xylose medium of the xylose fermented-yeasts isolated from sugarcane bagasses waste.

Sample Source	Isolated	Morphology Characteristics					Growth on xylose medium (OD 600 nm)	
		Form	Elevation	Surface	Margin	color	0 h.	48 h.
	KS 1-2	Irregular	Convex	Rough	Undulate	Cream	0.134	0.444
	KS 1-3	Circular	Convex	Glistening	Entire	Cream	0.057	0.833
	KS 1-4	Circular	Raised	Glistening	Entire	White	0.069	1.181
	KS 1-5	Circular	Convex	Glistening	Entire	Pink	0.044	0.319
	KS 2-1	Irregular	Raised	Glistening	Undulate	White	0.062	0.351
	KS 2-2	Circular	Convex	Glistening	Entire	White	0.056	0.684
	KS 2-3	Irregular	Convex	Glistening	Undulate	White	0.031	0.235
	KS 2-4	Circular	Convex	Glistening	Entire	White	0.052	0.793
	KS 3-1	Circular	Raised	Glistening	Entire	Cream	0.214	0.601
	KS 4-1	Irregular	Pulvinate	Glistening	Undulate	White	0.063	0.853
	KS 4-2	Circular	Flat	Glistening	Entire	Cream	0.078	0.908
	KS 4-4	Circular	Convex	Glistening	Entire	Pink	0.093	0.423
	KS 4-5	Filamentous	Flat	Rough	Lobate	White	0.046	0.489
	KS 4-6	Circular	Convex	Glistening	Entire	Pink	0.105	0.514
	KS 5-1	Irregular	Raised	Glistening	Lobate	White	0.109	0.406
	KS 6-1	Circular	Flat	Rough	Entire	White	0.066	0.555
	KS 6-2	Circular	Convex	Rough	Entire	White	0.090	0.618
	KS 7-1	Irregular	Convex	Rough	Undulate	White	0.082	0.467
	KS 7-2	Irregular	Flat	Glistening	Undulate	White	0.159	0.300
	KS 7-3	Irregular	Effuse	Rough	Undulate	White	0.086	0.680
	KS 7-4	Irregular	Convex	Rough	Undulate	White	0.096	0.589
	KS 7-5	Irregular	Flat	Rough	Lobate	White	0.094	0.669
	KS 7-6	Irregular	Flat	Glistening	Undulate	Yellow	0.057	0.242
	KS 7-7	Circular	Convex	Glistening	Entire	Pink	0.058	0.990
	KS 7-8	Irregular	Convex	Glistening	Lobate	White	0.059	0.266
	KS 7-9	Irregular	Pulvinate	Glistening	Undulate	White	0.083	0.539
	KS 8-1	Circular	Flat	Glistening	Undulate	White	0.068	0.197
	KS 9-1	Irregular	Convex	Glistening	Undulate	White	0.133	0.861
	KS 9-2	Filamentous	Raised	Rough	Filamentous	White	0.082	0.755
	KS 10-1	Rhizoid	Effuse	Glistening	Filamentous	White	0.068	0.493
	KS 10-3	Circular	Convex	Rough	Undulate	White	0.086	1.032
	KS 10-4	Circular	Pulvinate	Rough	Entire	White	0.097	0.691
Phu Wiang, Khonkaen Province	PV 1-1	Irregular	Convex	Glistening	Undulate	White	0.079	0.508
	PV 1-2	Circular	Raised	Glistening	Entire	Cream	0.223	0.864
	PV 1-4	Rhizoid	Convex	Rough	Filamentous	White	0.044	0.405
	PV 1-6	Circular	Convex	Glistening	Entire	Cream	0.069	0.907
	PV 2-1	Circular	Convex	Glistening	Entire	White	0.083	0.488
	PV 2-2	Circular	Pulvinate	Glistening	Entire	Cream	0.059	0.454
	PV 2-3	Circular	Convex	Glistening	Entire	Cream	0.057	0.180
	PV 3-1	Irregular	Pulvinate	Glistening	Undulate	White	0.082	1.280
	PV 3-3	Circular	Pulvinate	Glistening	Entire	Pink	0.095	0.414
	PV 3-4	Circular	Raised	Glistening	Entire	Cream	0.060	0.117
	PV 3-7	Circular	Convex	Glistening	Undulate	White	0.052	0.126
	PV 4-2	Circular	Pulvinate	Glistening	Entire	White	0.111	0.526
	PV 4-4	Circular	Convex	Glistening	Entire	White	0.087	0.541
	PV 4-5	Circular	Convex	Rough	Entire	White	0.097	0.566
	PV 5-1	Irregular	Flat	Glistening	Undulate	Cream	0.055	0.720

Table 3. Continued

Sample Source	Isolated	Morphology Characteristics					Growth on xylose medium (OD 600 nm)	
		Form	Elevation	Surface	Margin	color	0 h.	48 h.
	PV5-3	Filamentous	Pulvinate	Rough	Filamentous	White	0.120	0.513
	PV5-4	Irregular	Convex	Glistening	Undulate	Cream	0.120	0.241
	PV5-5	Rhizoid	Flat	Rough	Curld	Cream	0.030	0.040
	PV5-7	Rhizoid	Flat	Rough	Curld	Cream	0.027	0.190
	PV6-1	Circular	Convex	Glistening	Entire	White	0.097	0.623
	PV6-2	Circular	Convex	Glistening	Entire	White	0.062	0.485
	PV6-3	Circular	Convex	Rough	Entire	White	0.062	0.368
	PV6-7	Rhizoid	Pulvinate	Rough	Curld	Cream	0.041	0.335
	PV7-1	Circular	Convex	Rough	Entire	White	0.058	0.492
	PV7-4	Circular	Raised	Rough	Entire	White	0.083	0.319
	PV7-5	Circular	Convex	Rough	Entire	White	0.075	0.374
	PV8-1	Rhizoid	Pulvinate	Glistening	Curld	White	0.046	0.477
	PV8-3	Irregular	Flat	Rough	Undulate	White	0.048	0.362
	PV8-4	Circular	Convex	Rough	Entire	White	0.044	0.518
	PV9-1	Irregular	Raised	Rough	Undulate	White	0.026	0.344
	PV9-2	Circular	Pulvinate	Glistening	Entire	White	0.043	0.480
	PV9-3	Circular	Convex	Glistening	Entire	White	0.127	0.541
	PV9-4	Irregular	Convex	Glistening	Undulate	White	0.055	0.149
	PV9-5	Irregular	Flat	Rough	Undulate	White	0.045	0.328
	PV9-6	Circular	Convex	Glistening	Entire	Yellow	0.115	0.317
	PV10-3	Rhizoid	Convex	Rough	Filamentous	White	0.043	0.085
	PV10-4	Circular	Pulvinate	Rough	Entire	White	0.043	0.288

production of xylitol has the advantage of being a more attractive process, since its downstream processing is simple, and its fermentation process provides high cell density, thus resulting in high xylitol yields. Moreover, it is more economic and can be achieved in the industry without high pressure, temperature or xylose purification (Silva *et al*., 1999; Kang *et al*., 2005). Ideally microorganism xylitol producers are easy to cultivate, highly capable of productivity and have special resistance to pressure and toxins. According to recent research, the used of a variety of wild type yeast strains mostly genera *Candida* has shown potential production on an industrial scale including *C. boidinii* (Vandeska *et al*., 1995), *C. guilliermondii* (Zagustina *et al*., 2001; Rodrigues *et al*., 2003), *C. parapsilosis* (Oh *et al*., 1998), *C. peltata* (Saha *et al*., 1999) and *C. tropicalis* (Kim *et al*., 2002; López *et al*., 2004). Sirisansaneeyakul *et al.* (1995) selected a xylitol producer as *C. mogii* ATCC 18364 from 11 strains of D-xylose, utilizing yeasts they have screened in previous research. Their results indicated maximum xylitol yield of Yp/s = 0.62 g/g and a specific rate for product formation that was more than the other yeasts. Ikeuchi *et al.* (Ikeuchi *et al*., 1999) demonstrate microorganisms with the ability to produced xylitol from high concentrations of xylose were screened from 1,018 types of soil from farms and parks in Osaka, Japan by enrichment culture. A chemically defined (CD) medium using xylose as a sole carbon source for primary selection was obtained from yeast strain capable of metabolizing xylose and showed rapid growth on media containing xylose concentrations of 200 g/l. Results showed that 102 isolates and secondaries were selected using a semi defined (SD) medium with xylose concentration 250 g/l found that yeast 559-9 strain gave the maximum result at 204 g/l and this was identified as *Candida* sp. reference 'The Yeasts' (3rd edition) (Baz *et al*., 2011). In this strain, the biochemical and morphological characteristics are similar to *Candida* sp. Altamirano *et al.* (2000) isolated thirty-three yeast strain from natural resources, including corn silage (ASM strains) and viticulture residues (SJV strains) with growth on a medium with xylose as a carbon source. These strains were identified as *C. membranifaciens*, *C. tropicalis*, *C. guilliermondii*, *C. shehatae*, *Pichia capsulate*, *C. utilis*, *Candida* sp., *P. angusta* by C.P. Kurtzman, National Center for Agricultural Utilization Research (U.S.A.) and accessioned into ARS Culture Collection (NRRL) as NRRL Y-27290. The results of selected strains capable of producing xylitol showed that *C. tropicalis* has the highest potential to yield 0.69 g/g, using TLC techniques and HPLC. Kang *et al*. (2005) isolated a novel yeast strain from many kinds of soil from rice farming. The isolated strain was identified as *C. tropicalis* HY200 based on systematic characterization using general approaches of Biolog MicrologTM and

18S rRNA sequence analyses. This strain obtained high xylitol yield at 77% and a productivity amount of 2.57 g/l/h from 200 g/l of xylose concentration. Guo *et al*. (2006) explained the principles of xylitol producer screening for forty-five isolates, from 274 isolates grown on solids and broth with xylose as a carbon source. They comprised 9 genera. *Saccharomyces*, *Schizoblastosporion*, *Candida*, *Geotrichum*, *Pichia*, *Hansenula*, *Trichosporon*, *Sporobolomyces,* and *Rhodotorula*. Five species were selected for further experiments, toward the high utilization of xylose and D_1/D_2 of 26S rDNA identified as *C. guiliermondii* Xu280 and *C. maltosa* Xu316 which produced the highest xylose consumption and xylitol yield in batch fermentation under microaerobic conditions.

3.3 Xylose assimilation tests

Seventy-two isolates from sugarcane bagasse were tested for their abilities to assimilate xylose. All strains showed significant growth on liquid medium with xylose as the sole carbon source. Analysis of optical determination (OD600) showed that the strains, which grew well on xylose medium consumed xylose rapidly up to 48 hours. From these 72 strains, six strains including KS 1-4, KS 7-7, KS 10-3, PV 1-6, PV 3-1 and PV 5-1 were chosen for further study because of their high-growth rates on xylose medium. Results are shown in Table 3.

Previously different methods have been used for selecting the best xylitol producer yeasts from several strains, because different yeasts break down xylose at different rates because of species diversity. To ferment xylitol using all yeast species is difficult and it is necessary to find a method of selecting the best yeast culture to demonstrate the potential of using xylose as a carbon source. Altamirano *et al*. (2000) studied the isolation and identification of xylitol-producing yeasts from agricultural residues. Thirty-six yeast strains were primary screened for their capacities to convert D-xylose into xylitol using a conventional method by TLC adapted for easy determination of xylose and xylitol production. This technique is suitable for the first steps of a screening program to select xylitol-producing yeasts from natural environments. *C. tropicalis* ASM III (NRRLY-27290), isolated from corn silage had to high xylitol yield of 0.88 g xylitol/g of xylose consumed. Guo *et al*. (2006) accurately cultivated 274 strains on both solid and liquid screening media with xylose as the sole carbon resource. Five strains were selected based on significant high growth of assimilated xylose, which showed that the strains with rapid growth rate also consumed xylose rapidly. This method demonstrated that in the early testing it is easy to select varieties that produce high xylitol yields, and further study the conditions that affect xylitol production. Xylitol production using all yeast strains from isolation in the flask scale may be difficult, since it is time consuming, inconvenient to harvest and will increase the cost of the analysis. Therefore, this study used the primary screening method by Guo *et al*. (2006), which preliminary selected the yeast which used xylose quickly within 48 hours, observed from changes in the growth of yeast in the broth and significant turbidity increase. These strains were selected as inoculum for yeast fermentation of xylose in sugarcane bagasse hydrolysate to compare their ability to produce xylitol production, in the secondary screening of xyltitol-yeast producers from the xylitol yields.

3.4 Xylitol fermentation

Owing to their abilities to assimilate xylose rapidly, six strains were selected for further evaluation. Physiological behavior of these strains on hydrolysate fermentation medium (xylose concentration 32.30 g/l) was investigated. Xylitol production accompanied xylose consumption during yeasts growth. KS 10-3 exhibited the highest xylitol yield as shown in Table 4. The results in Table 4 show the six yeast strains (KS 1-4, KS 7-7, KS 10-3, PV 1-6, PV 3-1, and PV 5-1) primarily selected using xylitol fermentation of xylose in sugarcane bagasse hydrolysate compared to the reference strain *C. guilliermondii.* In batch fermentation was carried out with flask level (initial xylose 32.30 g/l) and time variation. Results showed that KS 10-3 strain gave maximum xylitol yield of 0.47 g/g with 96 hours at 30°C. Sequence D_1/D_2 of 26S rDNA analysis results identified this strain as *Candida tropicalis* KS 10-3 because 100% sequence identity is strain to that in database (length 576 nucleotides). 26S ribosomal RNA gene, partial sequence showed

AAACCAACAGGGATTGCCTTAGTAGCGGCGAGTGAAG
CGGCAAAAGCTCAAATTTGAAATCTGGCTCTTTCAGAG
TCCGAGTTGTAATTTGAAGAAGGTATCTTTGGGTCTGG
CTCTTGTCTATGTTTCTTGGAACAGAACGTCACAGAGG
GTGAGAATCCCGTGCGATGAGATGATCCAGGCCTATGT
AAAGTTCCTTCGAAGAGTCGAGTTGTTTGGGAATGCA
GCTCTAAGTGGGTGGTAAATTCCATCTAAAGCTAAATA
TTGGCGAGAGACCGATAGCGAACAAGTACAGTGATGG
AAAGATGAAAAGAACTTTGAAAAGAGAGTGAAAAA
GTACGTGAAATTGTTGAAAGGGAAGGGCTTGAGATCA
GACTTGGTATTTTGTATGTTACTTCTTCGGGGGTGGCCT
CTACAGTTTATCGGGCCAGCATCAGTTTGGGCGGTAGG
AGAATTGCGTTGGAATGTGGCACGGCTTCGGTTGTGTG
TTATAGCCTTCGTCGATACTGCCAGCCTAGACTGAGG
ACTGCGGTTTATACCTAGGATGTTGGCATAATGATCTT
AAGTCGCCCGTCT.

Moreover, this result shows that sugarcane bagasse as waste from the sugar factory is a good local alternative to use as a low cost substrate, with added value since residual xylose and other sugar especially xylose, is a substrate in xylitol production. Results agree with the Silva *et al*. (2011) study of glucose:xylose ratio effect to *C. guilliermondii* during fermentation of sugarcane bagasse and show that the glucose:xylose ratio of 1:5 has the highest xylitol yield at 0.59 g/g and demonstrates that glucose, arabinose and acetic acid all assist in the fermentation.

Xylitol production from sugarcane bagasse hydrolysate mostly using *C. guilliermondii* in Table 5 found to be

Table 4. Xylose fermentation of selected strains cultivated on a hydrolysate fermentation medium under micro-aerobic conditions. Initial xylose concentration was 32.30 g/l.

Strains	Culture time (h)	Sugar consumption (g/l)			$Y_{xl/s}$: Xylitol Yield (g xylitol/ g xylose consumed)
		xylose	glucose	arabinose	
KS 1-4	24	30.16	2.36	9.2	ND
	48	28.96	2.18	9.30	ND
	72	29.44	2.30	9.0	ND
	96	27.04	1.94	8.94	ND
KS 7-7	24	30.70	2.88	7.10	ND
	48	31.18	3.11	8.40	ND
	72	31.18	3.06	7.97	ND
	96	31.90	3.23	8.41	ND
KS 10-3	24	30.40	3.06	2.22	0.11
	48	2.66	1.14	6.90	0.14
	72	5.80	1.22	5.70	0.42
	96	1.50	0.28	4.14	0.47
PV 1-6	24	28.54	2.08	9.26	ND
	48	29.03	2.16	9.08	ND
	72	30.51	2.46	9.66	ND
	96	29.60	2.33	9.46	ND
PV 3-1	24	27.96	2.18	9.40	ND
	48	27.06	2.06	9.22	ND
	72	28.06	2.28	9.46	ND
	96	29.36	2.50	9.76	ND
PV 5-1	24	29.50	2.33	9.57	ND
	48	29.00	2.26	9.21	ND
	72	29.20	2.36	9.52	ND
	96	30.19	2.56	9.72	ND
C. guillier mondii	24	32.00	3.30	8.66	0.09
	48	32.36	3.36	7.24	0.11
	72	34.12	3.64	7.52	0.11
	96	32.94	3.48	7.32	0.11

ND = Not Detected

C. guilliermondii FTI 20037 high xylitol yields were 0.69, 0.75 and 0.81 g/g from xylose initial concentrations 30, 48, and 80 g/l, respectively. *C. guilliermondii* has a xylitol yield of 0.59 g/g from 45 g/l of xylose. *C. langeronii* RLJ Y-019 present xylitol yield 0.40 g/g from 47.2 g/l xylose concentration. Reports of recent research suggest that *C. guilliermondii* has a high potential of xylitol yield from sugarcane bagasse hydrolysate. Previous results show that *C. tropicalis* is a xylitol-yeast which produces a high xylitol yield similar to *C. guilliermondii*. Such findings suggest that the maximum xylitol yield of 0.45 g/g was achieved with the sugarcane bagasse hydrolysate but xylitol yields were lower with mixed sugar because of the limited yeast growth rate and inhibitor effect on cell adaptation (Rao *et al.*, 2006). Results are similar to these observed in xylitol production by Baz *et al*. (2011) using *C. tropicalis* under different condition such as rice bran, ammonium sulfate and xylose resulting in xylose giving a maximum xylitol yield of 57.2% obtained from xylose 20 g/l, rice bran 15 g/l and ammonium sulfate 1 g/l pH 5.5. However, xylitol increased to 72.5% when initial xylose was 50 g/l. Xylitol production on a large scale requires oxygen aeration by semi-synthetic media and hydrolysate. Using *C. tropicalis* AY2007 under a limit of O_2 0.3 vvm gave xylitol 36 g/l in 59 hours of fermentation and increase aeration of 1.5 vvm obtained 30.99 g/l. In comparison, xylitol yield from semi-synthetic media and hydrolysate were 0.704 and 0.783 g/g, respectively.

For this work, KS 10-3 gave the maximum xylitol yield at 0.47 g/g obtained at 32.30 g/l of xylose lower than some report in Table 5 towards initial xylose is low and necessary optimized condition suitable for this xylitol-yeast producer strain of xylitol production particularly xylose concentration and agitation rate influence cell yeast growth and increase xylitol production efficiency.

Table 5. Summary using sugarcane bagasse hydrolysate for xylitol production by genus *Candida*.

Yeast strain	Condition	Initial xylose	Yp/s (g/l)	Qp (g/g)	Time (g/l/hr)	Reference (hour)
C. guilliermondii FTI20037	Temperature at 30°C, agitation rate 300 rpm	48	0.75	0.57	22	Felipe *et al*., 1997
C. langeronii RLJ Y-019	Temperature at 42°C, agitation rate 700 rpm	47.2	0.40	0.97	-	Nigam, 2000
C. guilliermondii FTI20037	Temperature at 30°C, agitation rate 300 rpm	30	0.69	0.68	-	Martinez *et al*., 2003
C. guilliermondii	Temperature at 30°C, agitation rate 500 rpm	45	0.59	0.53	48	Silva *et al*., 2007
C. guilliermondii FTI20037	Temperature at 30°C agitation rate 200 rpm	80	0.81	0.60	48	Arruda *et al*., 2011
C. tropicalis	Temperature at 30°C agitation rate 200 rpm	56%	0.45	-	48	Rao *et al*., 2006
C. tropicalis	Temperature at 30°C agitation rate 200 rpm	50	36.25 g/l	-	96	Baz *et al*., 2011
C. tropicalis AY2007	Temperature at 30°C, agitation rate 200 rpm, aeration 0.3 vvm	29.8 45.5	0.783 0.704	0.239 0.506	94 65	Baz *et al*., 2011
C. tropicalis	Temperature at 30°C agitation rate 200 rpm	32.30	0.47	-	96	This study

4. Conclusions

In summary, we reported a xylose fermented-yeast had the ability to use xylose as a carbon source from sugarcane bagasse, KS 10-3 can grew well on xylose medium consumed xylose rapidly and produced a higher xylitol yield than *C. guilliermondii*. Therefore, a better understanding of the regulation of xylose metabolism will contribute to the design of xylose reductase and xylitol dehydrogenase activity to enhance the xylitol production and optimization that affect xylitol yield. Together with the complete genome sequence available from related strains, detailed genetic studies are expected to provide some clues for this information. This strain is expected to be a good candidate for studying xylitol production in large scale fermentation.

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