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Isolation of thermotolerant xylose-utilizing yeasts for ethanol and xylitol production

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ABSTRACT

Aims: The implementation of simultaneous saccharification and co-fermentation (SScF) and consolidated bioprocessing (CBP) is highly anticipated for industrial bioethanol applications. Thus, microorganisms capable of utilizing hexose and pentose sugars, as well as thermotolerant, are considered advantageous for optimum ethanol production.
Methodology and results: Thermotolerant yeast strains were isolated from wastewater ponds of ethanol-producing facility as well as empty fruit bunch composting area and screened for xylose- and glucose-fermenting ability. Five out of 24 total isolates were able to grow at 40 ºC and were found positive for ethanol production from xylose. Based on their high efficiency of xylose and glucose utilization, two isolates were chosen for further characterization. They were identified as *Kluyveromyces marxianus* UniMAP 1-1 and *Schwanniomyces etchellsii* UniMAP 1-7 based on the D1/D2 region of the large subunit ribosomal DNA. The growth kinetics of each isolate on xylose and glucose at 40 °C were determined. The two isolates were able to ferment xylose to ethanol at a maximum concentration between 0.533 ± 0.415 and 1.243 ± 0.246 g/L with concomitant xylitol production between 9.932 ± 0.303 and 12.933 ± 0.505 g/L. Fermentation of glucose to ethanol was also tested for these isolates and the yields were and 0.361 and 0.118 g/g for UniMAP 1-1 and UniMAP 1-7, respectively.
Conclusion, significance and impact of study: The potential of these thermotolerant microbes to be used for xylitol and bioethanol production from lignocelluloses are evident from this study.

Keywords: bioethanol, lignocellulosic biomass, thermotolerant, xylose-utilizing yeast, xylitol

INTRODUCTION

Lignocellulosic biomass is commonly referred to as residuals of agriculture, industry, and forest, which generally consists of 25% lignin and 75% sugars (Lorliam *et al*., 2013). Recently, lignocelluloses are becoming important due to their potential as abundant renewable carbon sources. Lignocelluloses consist of crystalline cellulose and hemicellulose which are tightly packed with amorphous lignin (Zhao *et al*., 2012). Hydrolysis of the cellulose and hemicellulose components resulted in hexose sugars, D-glucose, and pentose sugars, mainly D-xylose. However, fermentation of D-xylose by microorganisms, especially yeasts, is very challenging due to the cofactor imbalance between xylose reductase that prefers NADPH and xylitol dehydrogenase that has a higher affinity for NAD (Harner *et al*., 2015). Xylose-fermenting yeasts such as *Scheffersomyces stipitis*, *Pachysolen tannophilus*, *Scheffersomyces shehatae*, *Candida tenuis*, *Pichia segobiensis*, *Brettanomyces naardenensis*, *Spathaspora passalidarum*, and *Spathaspora arborariae* have been identified as capable of producing ethanol from xylose (Cadete *et al*., 2012; Lorliam *et al*., 2013). Besides ethanol, the main product of D-xylose reduction is a sugar alcohol known as xylitol that is used as an alternative sweetener. Unlike other natural sweeteners, xylitol is beneficial for dental health as it reduces caries and possesses a lower caloric value (Steinberg *et al*., 1992).

Microorganisms that have tolerance to elevated temperature normally serve as ideal workhorses in industrial practices, especially those that have broad substrate utilization, high fermentation rate, and tolerance to industrial inhibitors. Thermotolerant xylose-fermenting

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yeasts would therefore be highly valuable. *Kluyveromyces marxianus*, *Hansenula polymorpha*, *Pichia kudriavzevii*, and *Debaryomyces hansenii* are among the recently identified thermotolerant ethanologenic yeasts isolated from different sources (Banat and Marchant, 1995; Ryabova *et al*., 2003; Fonseca *et al*., 2008; Menon *et al.*, 2010; Dhaliwal *et al.*, 2011). The present study is aimed at isolating yeasts that are capable of fermenting xylose at elevated temperature from wastewater ponds of ethanol-producing facilities as well as oil palm mill waste areas. Isolates that exhibited the capability to utilize both xylose and glucose as carbon sources were characterized and their kinetics during exponential growth were studied. Finally, their xylose-fermentation and glucose-fermentation products and yields were determined.

MATERIALS AND METHODS

Isolation of xylose-utilizing yeasts

Samples were taken from the wastewater pond of Fermpro Sdn. Bhd. located at Perlis, Malaysia, and the empty fruit bunch composting area of Taclico Co. Sdn. Bhd., Kedah, Malaysia. Each sample (0.5 g) was mixed with 50 mL YPX medium (1% yeast extract, 2% peptone, and 2% D-xylose, w/v) supplemented with 0.02% chloramphenicol. The enriched samples were incubated at 40 °C for 3 – 10 days and subsequently spread on YPX agar for microbial isolation. Representative yeast colonies were selected, purified, kept on YPX agar at 4 °C, and stored in YM broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose and 1.5% agar, w/v) containing 10% (w/v) glycerol at −80 °C.

Morphology and biochemical characterization

Morphological characteristics were examined according to Kurtzman *et al*. (2011). Formations of true- and pseudo-hyphae were monitored in cornmeal agar at 25 °C for 7 days. The assimilation test was performed using the yeast identification kit API® 20C Aux (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's protocol. Identification was performed using api*web*™ identification software. The kit allows the evaluation of the assimilation of 20 carbon sources by yeast. Test strips were incubated at 30 °C for 48 to 72 h.

Molecular identification

The yeast isolates were identified based on the analysis of the D1/D2 region of their large subunit (LSU) of rDNA sequences. The amplified products of the genes were obtained by using the primer sets LR0R (5'-ACCCGCTGAACTTAAGC-3') and LR7 (5'-TACTACCACCAAGATCT-3') and sequenced at Macrogen Inc., Seoul, Korea. Sequences obtained were compared to sequences in the GenBank database (http://www.ncbi.nlm.nih.gov). Each sequence was aligned with related yeast sequences retrieved from GenBank using the multiple alignment program MUSCLE available within the software Molecular Evolutionary Genetics Analysis (MEGA) 6.0 (Hall, 2013). A phylogenetic tree was constructed using the Maximum Likelihood method with maximum composite likelihood correction based on the evolutionary distance. Phylogeny was tested by performing 1,000 bootstrap replications using the MEGA 6 software (Hall, 2013). Sequences determined in this study were deposited in the GenBank database and their accession numbers are shown in Table 1.

Table 1: Sampling area and identification of new isolates.

Sampling area	No. of isolates	Growth Temperature (°C)	Positive ethanologen in xylose	Strain	Identification	Similarities	GenBank accession no.
Wastewater Pond, Fermpro Sdn. Bhd, Bkt Keteri, Beseri, Perlis, Malaysia.	17	40	3	UniMAP 1-7	*Schwanniomyces etchellsii*	100%	KX538802
EFB compost area, Taclico Co. Sdn. Bhd, Kulim, Kedah, Malaysia.	7	40	2	UniMAP 1-1	*Kluyveromyces marxianus*	100%	KX538800
TOTAL ISOLATES	24		5				

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Growth profile determination and fermentation conditions

Each yeast strain was inoculated in 20 mL YPD medium (10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose) and incubated at 30 °C and 150 rpm for 24 h. These starter cultures were then transferred and grown aerobically in 200 mL YPD for 48 h at 30°C and 150 rpm. The cells were then centrifuged (Thermo Scientific) at 3000 × *g* for 10 min and washed twice with sterile distilled water. After pelleting, the cells were adjusted to 250 g/L of wet cells with distilled water and were ready to be inoculated for growth profile determination. The growth profile was determined in 100 mL of YP medium (10 g/L yeast extract and 20 g/L peptone) supplemented with 40 g/L of D-xylose or 40 g/L of D-glucose as substrate. The pH of the medium was adjusted to pH 4.5 with 0.5 M HCl. Inoculum culture with concentration of 10 g/L was inoculated into 100 mL of YPX medium and was shaken on a rotary shaker at 150 rpm at 40 °C for 96 h. Approximately, 2 mL of samples were withdrawn each time at 0, 3, 6, 9, 24, 48, 72, and 96 h. Cell growth was monitored by determination of biomass formation. All experiments were done in triplicates.

Analysis of substrate and products

The samples of cell culture taken out at 0, 3, 6, 9, 24, 48, 72, and 96 h were centrifuged at 4,000 rpm for 5 min. The supernatants were kept for analysis of xylose, glucose, ethanol, and xylitol concentrations by high-performance liquid chromatography (Shimadzu, Kyoto, Japan) with a refractive index detector. The mobile phase used was 5 mM H_2SO_4 with a flow rate of 0.5 mL/min. The column used was an Aminex® HPX-87H (BioRad) with the oven temperature set to 55 C.

RESULTS AND DISCUSSION

Isolation and characterization of xylose-utilizing yeasts

New yeast strains that can utilize xylose at an elevated temperature are industrially desirable and could potentially be exploited in fermentations. A total of 24 isolates that were able to grow at 40 °C were isolated from different samples collected from various sites (Table 1). The isolated strains were identified by cultural characteristics and growth pattern studies. Five of the yeast isolates were found to be positive for ethanol production from xylose. However, considering their ability to utilize both xylose and glucose, only two isolates were chosen for further characterization.

The two isolates were characterized morphologically and molecularly. The first isolate, UniMAP 1-1 formed a creamy white, circular and smooth colony when plated onto YPD agar. When observed under the light microscopy, UniMAP 1-1 cells have spheroidal, ovoid and occasionally cylindrical shape and formed budding. The second isolate, UniMAP 1-7 has a creamy white, smooth and butyrous colony on YPD agar. The UniMAP 1-7 cells were observed to have spheroidal to ovoid shape and formed budding under the light microscopy. The isolated strains were then identified based on the analysis of their D1/D2 region of LSU rDNA. The two isolates, UniMAP 1-1 and UniMAP 1-7, were identified as *Kluyveromyces marxianus* UniMAP 1-1 and *Schwanniomyces etchellsii* UniMAP 1-7, respectively. The partial sequences of D1/D2 gene regions of *Kluyveromyces marxianus* UniMAP 1-1 and *Schwanniomyces etchellsii* UniMAP 1-7 were submitted to GenBank with accession numbers KX538800 and KX538802, respectively. Table 1 and Figure 1 summarized the characteristics of these isolates.

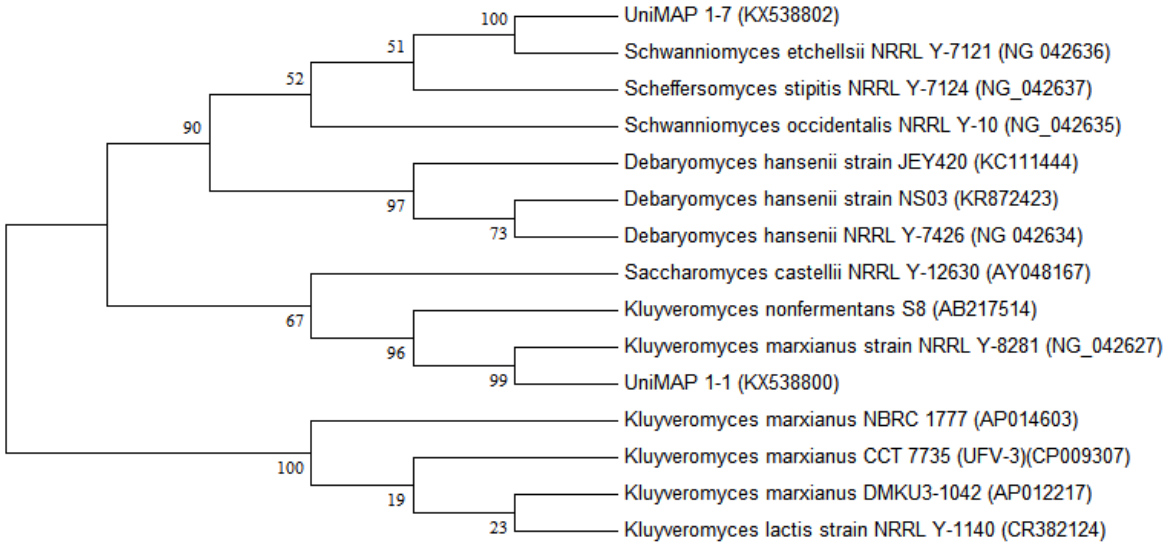

Figure 1: Phylogenetic tree of xylose assimilation yeasts constructed by the Maximum Likelihood method based on the D1/D2 domain of LSU rRNA gene sequences. Numbers represent the percentages from 1,000 replicate bootstrap resamplings.

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Their sugar assimilation capability was also tested. The UniMAP 1-1 strain assimilated D-glucose, glycerol, L-arabinose, D-xylose, adonitol, xylitol, D-galactose, D-sorbitol, D-saccharose, and D-raffinose (Table 2). It has almost similar phenotypic characteristics with the type strain *Kluyveromyces marxianus* NRRL Y-8281^T (Kurtzman *et al*., 2011). This isolate is also closely related to *Kluyveromyces marxianus* NRRL Y-8281^T based on the identical nucleotide sequence of the D1/D2 domain of the 26S rRNA gene (Table 1, Figure 1).

The isolate UniMAP 1-7 is closely related to *Schwanniomyces etchellsii* NRRL Y-7121 based on the nucleotide sequence of the D1/D2 domain of the 26S rRNA gene. This species is also clustered together with *Debaryomyces hansenii* (Figure 1) which is biotechnologically important due to its osmo- and xerotolerance as well as halotolerant extremophilic characteristics (Breuer and Harms, 2006). Isolate *S. etchellsii* UniMAP 1-7 assimilated D-glucose, glycerol, calcium-2-keto-gluconate, D-xylose, adonitol, D-galactose, D-sorbitol, methyl-αD-glucopyranoside, N-acetyl-glucosamine, D-maltose, D-saccharose and D-melezitose (Table 2). These phenotypic characteristics are similar to the type strain described by Kurtzman *et al*. (2011).

Table 2: Assimilation of sugars for new strains isolated.

Characteristics	*K. marxianus* UniMAP 1-1	*S. etchellsii* UniMAP 1-7
Assimilation of:		
D-Glucose	+	+
Glycerol	+	+
Calcium 2-Keto-Gluconate	-	+
L-Arabinose	+	-
D-Xylose	+	+
Adonitol	+	+
Xylitol	+	-
D-Galactose	+	+
Inositol	-	-
D-Sorbitol	+	+
Methyl-αD-Glucopyranoside	-	+
N-Acetyl-Glucosamine	-	+
D-Cellobiose	-	-
D-Lactose	-	-
D-Maltose	-	+
D-Saccharose	+	+
D-Trehalose	-	-
D-Melezitose	-	+
D-Raffinose	+	-

Growth using xylose and glucose as the sole carbon source

The newly isolated strains, UniMAP 1-1 and UniMAP 1-7 were cultivated aerobically in YP medium containing either 4% xylose or glucose at 40 °C. Figure 2 depicts the growth and sugar consumption profiles of the two newly isolated yeasts. Growth of *K. marxianus* UniMAP 1-1 in xylose was most rapid for the first 9 h of fermentation. After 9 h, the growth of UniMAP 1-1 was gradual and CDW (cell dry weight) peaked at 72 h at 7.1 g/L before decreasing to 6.6 g/L at 96 h. On the other hand, the growth of *S. etchellsii* UniMAP 1-7 in xylose was in lag phase for the first 9 h of fermentation. The growth of *S. etchellsii* UniMAP 1-7 steadily increased and reached a maximum biomass of 8.0 g/L CDW at 72 h (Figure 2a).

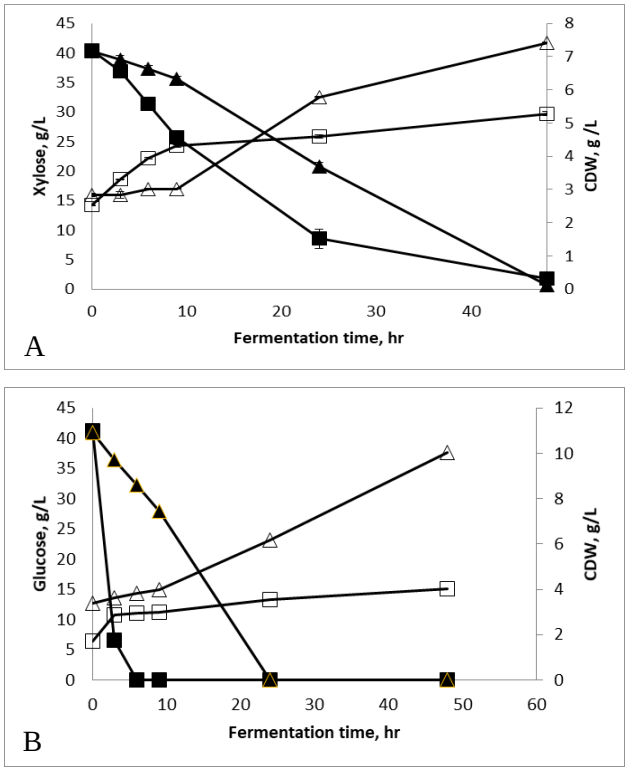

Figure 2: Growth and sugar consumption profile of new isolates on (a) D-xylose and (b) D-glucose at 40°C, the growth of isolates is represented by empty symbols (□) *K. marxianus* UniMAP 1-1; (△) *S. etchellsii* UniMAP 1-7; xylose or glucose consumption is represented by filled symbols (▯) *K. marxianus* UniMAP 1-1; (▲) *S. etchellsii* UniMAP 1-7.

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For all isolates, 40 g/L xylose was supplemented in the culture medium as the sole carbon source. Figure 2a shows that xylose was continuously consumed by these isolates during their growth and the consumption of xylose was completed at 48 h of fermentation.

In comparison, the growth of isolate UniMAP 1-1 was observed to be more rapid during glucose fermentation. Isolate *K. marxianus* UniMAP 1-1 was able to completely consume the provided glucose after 6 h of fermentation (Figure 2b). The efficiency of glucose consumption for this strain was reflected by its rapid growth. The growth profiles of *K. marxianus* UniMAP 1-1 increased sharply for the first 6 h. The amount of biomass for *K. marxianus* UniMAP 1-1 increased 1.7 folds from 1.7 g/L CDW to 2.9 g/L CDW before it reached stationary phase. The growth of *S. etchellsii* UniMAP 1-7 in glucose, on the other hand, slowly increased and cell density reached its maximum at 96 h at 10.0 g/L CDW. Its consumption of glucose was completed at 24 h of fermentation.

In general, all of these isolates are capable of growing on both xylose and glucose as a carbon source at 40 C. The growth parameters during exponential growth of the isolates were calculated and tabulated in Table 3. Comparing all experiments performed with xylose as carbon source at 40 C, the specific growth rate, μ_{max}, was highest for isolate *K. marxianus* UniMAP 1-1 with a value of 0.075 h^{-1} followed by *S. etchellsii* UniMAP 1-7 with μ_{max} of 0.022 h^{-1}.

The growth rate of the isolates in glucose at 40 C, on the other hand, was more rapid compared to their growth rate in xylose at the same temperature. With glucose as the carbon source, the growth rate of *K. marxianus* UniMAP 1-1 was highest with a μ_{max} value of 0.206 h^{-1}. On the other hand, the growth rate of *S. etchellsii* UniMAP in glucose had a μ_{max} value of 0.023 h^{-1} and this was surprisingly insignificantly different compared to its growth in xylose. This is in agreement with the physiological description of its type strain by Kurtzman *et al*. (2011) in that this species has a weak capability of fermenting glucose although it has a broad sugar assimilation capability (Kurtzman *et al*., 2011).

The specific growth rates for *K. marxianus* were reported to be between 0.214 - 0.45 h^{-1} at various temperatures after optimization during glucose fermentation (Kumar *et al.*, 2009; Fonseca *et al*., 2013; Arora *et al*., 2015) . Parameters optimization plays an important role in influencing the growth of microorganisms. Parameters such as growth temperature, initial pH, inoculum size and substrate concentration are among the reported parameters that affects growth directly (Charoenchai *et al*., 1998; Manikandan and Viruthagiri, 2010; Arora *et al*., 2015). Hence, the growth rate of these newly isolated yeasts are suggested to be improved through optimization of physical and chemical parameters that controlling their growth.

The specific rate of substrate consumption (μ_S) was calculated from the slope of *X* (biomass concentration) versus substrate concentration (*S*) plot only including data points belonging to the exponential growth phase (Fonseca *et al*., 2007). The μ_S values for cultivation in glucose at 40°C of isolated strains *K. marxianus* UniMAP 1-1 and *S. etchellsii* UniMAP 1-7 were rapid at rates of 3.459 g/gh and 0.341 g/gh respectively. Conversely, the substrate consumption rate in xylose at the same temperature was much slower at 0.638 g/gh for *K. marxianus* UniMAP 1-1 and 0.176 g/gh for *S. etchellsii* UniMAP 1-7. The growth rate and substrate consumption rate on xylose and glucose indicated that xylose uptake was less efficient than glucose in all of the isolates. *Saccharomyces cerevisiae*, for example, adopted the facilitated diffusion method using hexose transporter as the mediator for xylose transport. Hexose transporters have a high K_m value for xylose in *S. cerevisiae*, indicating that xylose uptake through these transporters is significantly less efficient compared to glucose (Chu and Lee, 2007). In another xylose-uptake experiment, *S. cerevisiae* cells demonstrated that its monosaccharide transport system had a nearly 200-fold lower affinity for xylose than for glucose, thus causing an inefficiency of xylose uptake in yeasts compared to glucose (Hamacher *et al*., 2002), explaining the inefficiency of xylose transport system in yeasts.

In all of the experiments performed, it is observed that the biomass yield ($Y_{x/s}$) obtained from xylose fermentation is higher than the biomass obtained in glucose fermentation (Table 3). This indicated that xylose was mainly used for growth instead of fermentation. As observed in Crabtree-positive yeasts such as *S. cerevisiae,* the reduction of biomass and the accumulation of ethanol is expected. During fermentation, the respiratory genes were repressed by the presence of glucose and the overflowing of carbon flux at the pyruvate level enhanced alcohol formation (Castrillo and Ugalde, 1993; Sonnleitner and Käppeli, 1986). This can be easily observed by the lower amount of biomass yield when *S. cerevisiae* is cultivated on glucose during batch fermentation (Van Dijken *et al*., 2000). Hence, explains the high amount of biomass obtained after xylose-fermentation compared to glucose fermentation.

Production of ethanol and xylitol by the newly isolated strains

Fermentation by these newly isolated strains was done in YP media supplemented with 40 g/L xylose or glucose at 40 °C to analyze product formation at higher temperature. *K. marxianus* UniMAP 1-1 and *S. etchellsii* UniMAP 1-7 were able to ferment glucose to ethanol, and xylose to both ethanol and xylitol. Fermentation of glucose produced the highest amount of ethanol for all isolates compared to fermentation of xylose. As shown in Table 3, *K. marxianus* UniMAP 1-1 produced a maximum of 14.869 g/L ethanol after 6 h of fermentation and this corresponded to a yield of 0.361 g/g. The least ethanol production was by *S. etchellsii* UniMAP 1-7 with 4.848 g/L of ethanol after 24 h with a $Y_{e/s}$ value of 0.118 g/g. Fermentation with xylose, on the other hand, produced xylitol as well as a small amount of ethanol. Based on the growth kinetics on xylose, all of the isolates were able to

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Table 3: Growth kinetics and fermentation parameters of isolate cultivated in D-xylose and D-glucose at 40 °C under aerobic condition.

Type of sugar	Isolate	Initial substrate conc (g/L)	Time for max. ethanol (h)	Max. ethanol conc. (g/L)	Max. xylitol conc. (g/L)	Specific growth rate, (μ_{max}/h)	$Y_{x/s}$ (g/g)	Specific substrate consumption rate, μ_s (g/gh)	$Y_{e/s}$ (g/g)	$Y_{xy/s}$ (g/g)
Glucose	UniMAP 1-1	40	6	14.869 ±0.224	ND	0.2062 ± 0.007	0.0596 ± 0.001	3.459	0.361	NA
	UniMAP 1-7		24	4.848 ± 0.350	ND	0.0234 ± 0.0002	0.0686 ± 0.001	0.341	0.118	NA
Xylose	UniMAP 1-1	40	24	1.243 ± 0.246	9.932 ± 0.303	0.0747 ± 0.005	0.117 ± 0.008	0.638	0.039	0.312
	UniMAP 1-7		48	0.533 ± 0.415	12.933 ± 0.505	0.0219 ± 0.0007	0.1243 ± 0.003	0.1762	0.013	0.326

ND Not detected, $Y_{x/s}$ biomass yield on substrate, $Y_{e/s}$ ethanol yield, $Y_{xy/s}$ xylitol yield.

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consume this carbon source within 48 h of fermentation (Figure 2). The amount of ethanol produced by *K. marxianus* UniMAP 1-1 was highest after 24 h at 1.243 g/L ethanol while *S. etchellsii* UniMAP 1-7 produced maximum ethanol after 48 h, which was 0.533 g/L (Table 3).

These newly isolated strains produced higher xylitol than ethanol during xylose fermentation. The strain *S. etchellsii* UniMAP 1-7, which generated the lowest concentration of ethanol, produced the highest concentration of xylitol after 48 h of fermentation with 12.933 g/L of xylitol and $Y_{xy/s}$ of 0.326 g/g. *K. marxianus* UniMAP 1-1 produced 9.932 g/L xylitol with $Y_{xy/s}$ of 0.312 g/g. The conceivable explanation for the accumulation of xylitol in the fermentation medium instead of ethanol in these isolated yeasts could be the occurrence of cofactor imbalance in the first two enzymes of xylose metabolism; xylose reductase (XR) has a higher affinity for NADPH while xylitol dehydrogenase (XDH) is only active with NAD (Harner *et al*., 2015). This cofactor imbalance inhibits further metabolism along the pathway, hence resulting in xylitol accumulation.

In this study, small amount of ethanol were produced from xylose by the newly isolated yeast strains compared to *S. stipitis* JCM 10742^T that yielded 0.285 g/gxylose, *S. passalidarum* yielded 0.4 g/g xylose, and *Zygoascus meyerae* E23 that was reported to yield ethanol between 0.017-0.08 g/g xylose. However, the cultivation temperatures of these yeasts were performed at normal temperature between 30-32 ˚C (Hou, 2012; Lorliam *et al*., 2013). As for xylitol production, Barbosa *et al*., (1988) reported a yield of 0.54 g/g xylose when *Candida guilliermondii* FTI-20037 was cultivated at 40.6ºC (Barbosa *et al*. 1988). Other than that, *Candida tropicalis* A26, *C. tropicalis* A12, *C. guilliermondii* Xu280 and *C. maltose* Xu316 were also reported to have high xylitol yield between 0.43 to 0.71 g/g at 30 °C (Guo *et al*., 2006; Lorliam *et al*., 2013).

Many different types of thermotolerant yeasts were also explored for their ability to produce ethanol from glucose and xylose. The methylotrophic yeast *Hansenula polymorpha* was able to grow at an elevated temperature of up to 48 C (Ryabova *et al*., 2003). Kim *et al*. (2013) reported three strains of *H. polymorpha* (strains DL-1, NCYC495 and CBS4732) for their ability to produce ethanol from both glucose and xylose at 37 °C. All of the three strains completely consumed 20 g/L of glucose between 12 to 48 h, producing 4 – 8 g/L of ethanol. These strains were able to consume 20 g/L of xylose within 24 h, accumulating xylitol mainly and trace amounts of ethanol at concentrations of 2.9 – 7.2 g/L and 0.1 – 0.5 g/L, respectively. Arora *et al*. (2015) isolated two thermotolerant strains identified as *K. marxianus* NIRE-K1 and NIRE-K3, and both were able to produce ethanol from glucose and xylose at 45 °C. In YP medium supplemented with 20 g/L glucose, both *K. marxianus* NIRE-K1 and NIRE-K3 were able to consume sugar completely in 16 h, producing maximum ethanol with yields of 0.31 and 0.36 g/g, respectively. In medium supplemented with xylose (20 g/L), ethanol was produced with concomitant xylitol production. *K. marxianus* NIRE-K1 produced maximum ethanol and xylitol concentration of 0.3 g/L and 4.34 g/L, respectively after 24 h of fermentation. Similarly, with the same duration of fermentation, *K. marxianus* NIRE-K3 accumulated 0.08 g/L of ethanol and 0.8 g/L of xylitol (Arora *et al*., 2015).

Additionally, it is interesting to note that this is the first report on the capability of *S. etchellsii* in producing ethanol and xylitol at high temperature. Previously, *S. etchellsii* or formerly known as *D. etchellsii* and *P. etchellsii* has been reported to exhibit a high levels of thermostable ß-glucosidases (BGLI and BGLII) activity (Pandey and Mishra, 1997). ß-glucosidases enzymes are important enzymes assisting in the cellulose hydrolysis for efficient ethanol production from lignocellulosic substrates (Wallecha and Mishra, 2003). Therefore, further studies are required to explore the potential of this yeast species to be used in the consolidated bioprocessing or simultaneous saccharification and fermentation (SSF) for bioethanol production.

CONCLUSION

This study has successfully isolated new yeast strains from a wastewater pond of an ethanol production facility and an oil palm mill waste area. These strains demonstrated the ability to ferment both glucose and xylose at an elevated temperature. The new isolates, *K. marxianus* UniMAP 1-1 and *S. etchellsii* UniMAP 1-7, demonstrated the ability to ferment glucose to produce ethanol, and xylose to produce both ethanol and xylitol. Each isolate has the desirable traits to be explored as a potential ethanol or xylitol producer in the future. Further studies on their physiology using other carbon sources such as lignocellulosic hydrolysates would be very appealing to assess their biotechnological potentials.

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