CHAPTER IV
BIOETHANOL PRODUCTION FROM JERUSALEM ARTICHOKE TUBERS JUICE BY THERMOTOLERANT YEAST *KLUYVEROMYCES MARXIANUS* DBKKU Y-102

4.1 Introduction

Ethanol production of fuel alcohol from renewable biomass is an attractive source of energy because plant biomass is the only sustainable source of organic fuels, chemicals, and materials available to humanity. Bioethanol can be made from various sources of carbohydrate materials that contain sugar and starch such as sugarcane, potato and corn. Jerusalem artichoke (*Helianthus tuberosus* L.) is one of the most interesting materials among traditional agricultural crops. This plant has many advantages over the conventional crops include the following: (a) minimal fertilizer requirement, (b) resist many plant pests and diseases, (c) high tolerance to frost and drought, and (d) very high carbohydrate yield per acre (Chubey and Dorell, 1974; Dorell and Chubey, 1977; Swanton et al., 1992). It belongs to the sunflower family, which can grow in a wide variety of climates. This plant contains nearly 20% of carbohydrates, 70–90% of which is inulin. Inulin is a polyfructan consists of linear chains of β (2→1) linked D-fructose units. Each chain is terminated by a D-glucose residue linked to fructose by an α (1→2) bond (Ge and Zhang, 2005; Szambelan et al., 2005), which has potential for ethanol fermentation, fructose syrup production, single cell oil and inulooligosaccharide (IOS) production (Chi et al., 2009; Zhao et al., 2010). Ethanol fermentation process at high temperature with thermotolerant yeasts have several advantages such as energy saving in cooling system, reduce risk of contamination, and increase the speed of catalytic reactions related to fermentation. There are several researches have been reported of effective of thermotolerant yeast strains capable of growing and producing ethanol at a temperature higher than 35ºC (Benjaphokee et al., 2012; de Souza et al., 2012; Hashem et al., 2013). Ethanol production efficiency from various materials depends on many factors such as initial pH of the medium, initial cell concentration and strain of the microorganism use for

fermentation. In this work, selection and characterization of thermotolerant yeasts *Kluyveromyces marxianus* capable of producing ethanol from Jerusalem artichoke (*Helianthus tuberosus* L.) were investigated. The influence of fermentation parameters on ethanol production such as pH of fermentation medium, initial sugar concentration, cell concentration, nitrogen source and concentration of magnesium sulfate on ethanol production by the selected yeast strain was also described.

4.2 Materials and methods

4.2.1 Microorganisms

The *Kluyveromyces marxianus,* strain DBKKU Y-102, DBKKU Y-103, DBKKU Y-104, DBKKU Y-105, DBKKU Y-106 and DBKKU Y-107, were isolated from various sources of samples such as sugarcane juice, decayed fruits and materials from Jerusalem artichoke plantation in Thailand. Pure cultures were maintained on yeast extract malt extract (YM) medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 1% glucose) and stored at 4^{o}C with subculturing every 2 months.

4.2.2 Inoculum preparation

All strains of *K. marxianus* were inoculated into a 250-ml Erlenmeyer flask containing 50 ml YM broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 1% glucose). The flask was incubated on a rotary shaker at 30ºC, with shaking at 200 rpm for 15 h. To increase cell concentration, the culture was transferred into a 500-ml Erlenmeyer flask containing 300 ml of the YM medium containing 100 g/l of glucose to give the initial cell concentration of 1×10^{6} cells/ml. The flasks were further incubated on a rotary shaker at 35ºC, with shaking at 200 rpm. After 15 h of incubation, the cells were harvested and used as an active inoculum for ethanol production.

4.2.3 Raw material

Jerusalem artichoke tubers (cultivar KKUAC001) were obtained from the Plant Breeding Research Center for Sustainable Agriculture, Faculty of Agriculture, Khon Kaen University, Thailand. The tubers were cleaned with tap water and ground into a mash using a food grinder. The juice were collected after pressing and kept at -20°C until use.

4.2.4 Ethanol production from Jerusalem artichoke juice

Ethanol fermentation ability of the isolated yeasts in Erlenmeyer flask was compared using Jerusalem artichoke juice without acid or enzymatic pre-treatment as a raw material. Batch ethanol fermentation was performed in 500 ml with 250 ml working volume. The flasks were inoculated with active inoculums at an initial concentration of 1×10^6 cells/ml under shaking speed at 100 rpm and incubated at 30, 37, 40 and 45^{o}C. During fermentation, samples were withdrawn at certain time intervals for further analysis. The relatively high ethanol producing strain of the isolated yeast was selected based on its growth and ethanol production performances at elevated temperatures.

4.2.5 Ethanol fermentation and optimization conditions

Sterilized of Jerusalem artichoke juice at 110°C for 28 min was directly used as the fermentation medium for ethanol production by *K. marxianus*. The effect of initial pH of fermentation medium was varied at 4.0, 4.5, 5.0, 5.5 and 6.0. The effects of sugar concentrations (230, 250 and 270 g/l) and initial cell concentrations (1×10^6, 1×10^7 and 1×10^8 cells/ml) on ethanol production were also determined. The effect of inorganic and organic nitrogen sources was evaluated. Inorganic nitrogen sources including ammonium sulfate and diammonium phosphate were used as supplementary nitrogen sources at 0, 0.25, 0.50, 0.75 and 1.00 g/l. Yeast extract (at the concentration of 6, 9 and 12 g/l) and corn steep liquor (at the concentration of 20, 30 and 40 g/l) were used as an organic nitrogen sources. The effect of concentrations of magnesium sulfate on ethanol production was studied at 0, 0.50, 1.00, 1.50 and 2.00 g/l. During fermentation, samples were withdrawn at certain time intervals for further analysis. All the experiments were replicated twice and the results were expressed as mean ±SD.

4.2.6 Batch fermentation for ethanol production in a jar fermenter

Ethanol fermentations were carried out in a 2L fermenter with a 1.2L working volume under the optimal conditions with an agitation speed of 100 rpm at 37ºC. A Jerusalem artichoke medium composed of the optimal pH level, concentration of sugar, cell number, nitrogen source, and concentration of magnesium sulfate on ethanol production as determined in flask scale was applied. During fermentation, samples were withdrawn at certain time intervals for further analysis.

4.2.7 Analytical methods

Total sugars were assayed by the phenol sulfuric acid method (Dubois et al., 1956). The yeast cell numbers and total soluble solids of the fermentation broth were determined by direct counting method using haemacytometer and hand-held refractometer, respectively (Zoecklien et al., 1995). The pH was measured by pH meter. Ethanol concentration in the culture medium was measured by gas chromatography (GC) (Shimadzu GC-14B, Japan) using polyethylene glycol (PEG-20M) packed column with a flame ionization detector. N_2 was used as a carrier gas and isopropanol was used as an internal standard (Laopaiboon et al., 2009). The ethanol yield (*Y*ps) was calculated as the actual ethanol produced and expressed as g ethanol per g sugar utilized (g/g). The volumetric ethanol productivity (*Qp*, g/l.h) was calculated by the following equations: $Qp = P/t$

Where P is the ethanol concentration (g/l) and t is the fermentation time (h) giving the highest ethanol concentration.

All the experiments were performed in duplicate and the results were expressed as mean ± SD of the duplicated experiments. The means were analyzed by Univariate using SPSS 15.0 for Windows program (SPSS Inc., 2006) with the general linear model procedure. DUNCAN test for multiple comparisons of the means was used for judging the significance of difference at the probability, $p \leq 0.05$.

4.3 Results and discussion

4.3.1 Comparison of growth and batch ethanol production

The comparative studies on growth of *K. marxianus* isolated in this study, i.e., DBKKU Y-102, DBKKU Y-103, DBKKU Y-104, DBKKU Y-105, DBKKU Y-106 and DBKKU Y-107 at various temperatures were examined and the results are shown in Figure 4.1. When grown in YM agar plates at 30, 37, 40 and 45°C, all strains capable of growing up to 45°C. Based on the description of thermotolerant microorganism given by McCracken and Gong (1982), the type strains were classified as thermotolerant yeast since their maximum temperatures ranging from 37-45°C.

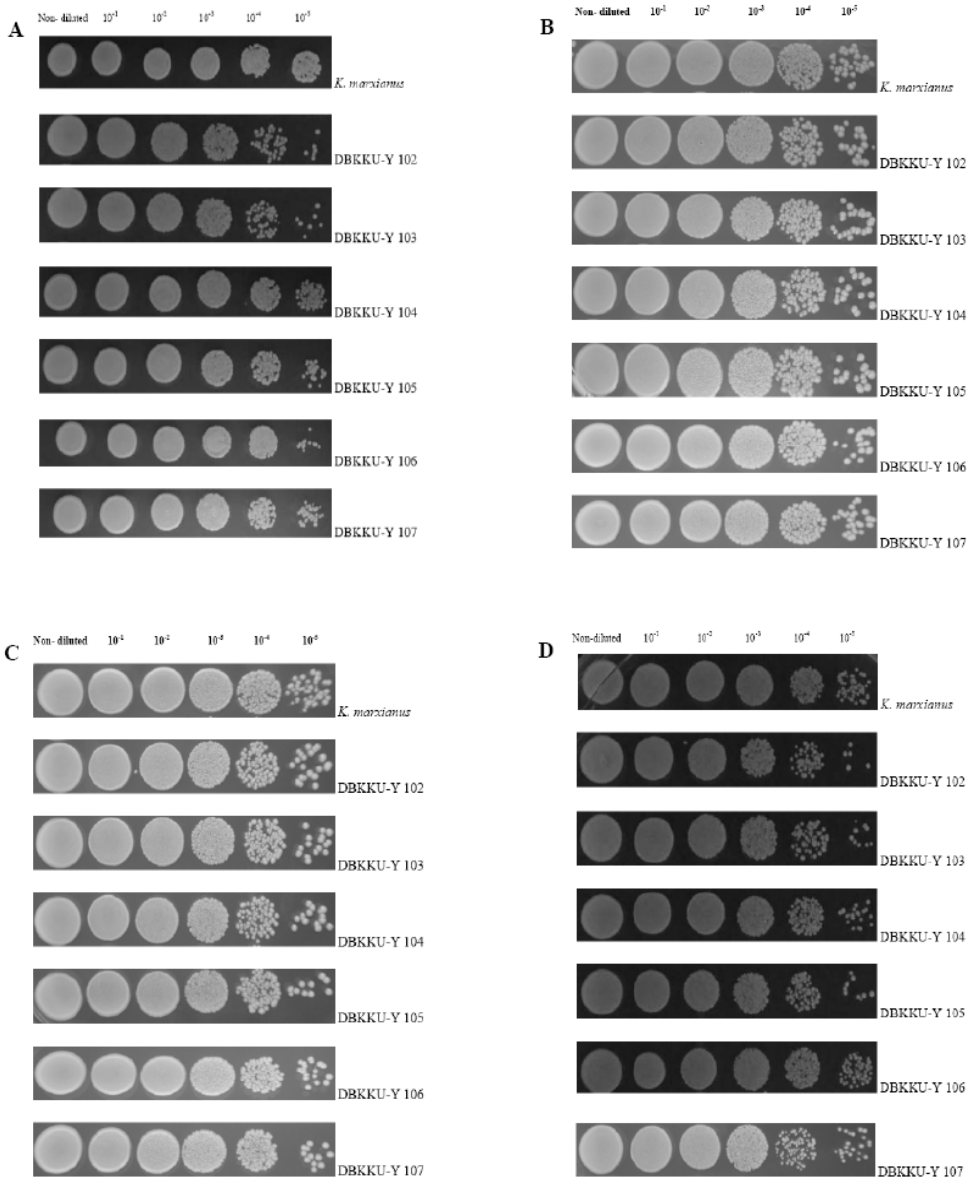

Figure 4.1 Exponentially growing yeast cultures of *K. marxianus,* DBKKU Y-102, DBKKU Y-103, DBKKU Y-104, DBKKU Y-105, DBKKU Y-106 and DBKKU Y-107 were spotted in 10-fold serial dilutions onto YM agar plates and grown at 30 (A), 37 (B), 40 (C) and 45°C (D) for 24 h

4.3.2 Ethanol production from Jerusalem artichoke juice by the isolated yeasts

Among the isolated strains, *K. marxianus* DBKKU Y-102 gave relatively high ethanol concentration at 37 and 40°C. The ethanol production profiles of these six isolated strains are shown in Figure 4.2. The results from this study indicated that increasing the fermentation temperature of all strains to 45°C resulted in drastically decreased in maximal ethanol concentrations and productivities (Table 4.1). Based on these results, the *K. marxianus* DBKKU Y-102 was selected as a high potential ethanol producing strain for further experiments, since it gave the highest ethanol concentrations and volumetric ethanol productivities were achieved at 30, 37 and 40°C. During ethanol fermentation particularly in tropical and non-tropical countries, the temperature may raise up to 40ºC due to combination of high average day time temperatures and exothermic metabolic reaction of yeast during active growth (Limtong et al., 2007; Yuangsaard et al., 2012). Therefore, the ethanol fermentation from Jerusalem artichoke will be carried out at 37 and 40ºC.

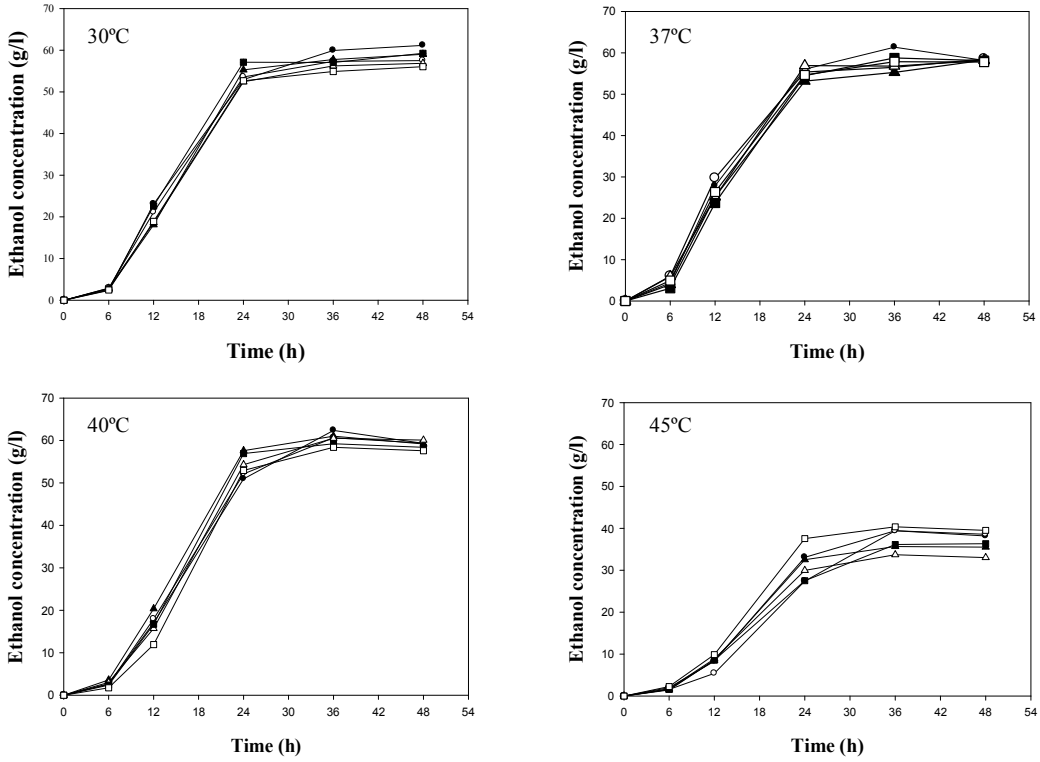

Figure 4.2 Ethanol production by DBKKU Y-102 (●), DBKKU Y-103 (○), DBKKU Y-104 (▲), DBKKU Y-105 (△), DBKKU Y-106 (■), and DBKKU Y-107 (□) in a Jerusalem artichoke juice under shaking speed at 100 rpm and incubated at 30, 37, 40 and 45^{o}C

Table 4.1 Comparison of ethanol fermentation by the six isolates of yeast in Jerusalem artichoke juice at various temperatures

Strain	Temperature (°C)							
	30		37		40		45	
	P **(g/l)**	***Qp*** **(g/l.h)**	***P*** **(g/l)**	***Qp*** **(g/l.h)**	***P*** **(g/l)**	***Qp*** **(g/l.h)**	***P*** **(g/l)**	***Qp*** **(g/l.h)**
102	59.94±0.18^{a} (36 h)	1.67±0.01^{a}	61.35±0.16^{a} (36 h)	1.70±0.00^{a}	62.38±0.18^{a} (36 h)	1.73±0.01^{a}	39.44±0.21^{a} (36 h)	1.10±0.01^{a}
103	57.35±0.36^{b} (36 h)	1.59±0.01^{b}	58.51±0.28^{b,c} (48 h)	1.22±0.01^{b}	60.64±0.16^{b,c} (36 h)	1.68±0.00^{b}	39.44±0.25^{a} (36 h)	1.10±0.01^{a}
104	59.01±0.35^{c} (48 h)	1.23±0.01^{c}	58.22±0.35^{b,c} (48 h)	1.21±0.01^{b}	61.01±0.21^{c} (36 h)	1.69±0.01^{b}	35.66±0.23^{b} (36 h)	0.99±0.01^{b}
105	56.23±0.25^{d} (36 h)	1.56±0.01^{d}	58.14±0.21^{b,c} (48 h)	1.21±0.00^{b}	60.53±0.24^{b} (36 h)	1.68±0.01^{b}	33.71±0.25^{c} (36 h)	0.94±0.01^{c}
106	59.26±0.44^{a,c} (48 h)	1.23±0.01^{c}	58.75±0.51^{c} (36 h)	1.63±0.01^{c}	59.62±0.10^{d} (36 h)	1.66±0.00^{c}	36.15±0.21^{b} (36 h)	1.00±0.01^{b}
107	56.06±0.06^{d} (48 h)	1.17±0.00^{d}	58.85±0.24^{b} (48 h)	1.61±0.01^{d}	58.41±0.10^{e} (36 h)	1.62±0.00^{d}	40.36±0.27^{d} (36 h)	1.12±0.01^{d}

P, ethanol concentration produced (g/l); *Qp,* volumetric ethanol productivity (g/l.h).

$^{a-e}$ Means followed by the same letter within a same column are not significantly different using Duncan's multiple range test at the level of 0.05.

The results were expressed as mean ±SD.

4.3.3 Optimization for ethanol fermentation by *K. marxianus*

The effect of initial pH of Jerusalem artichoke juice medium was investigated, and the results showed that at 37 and 40°C, the ethanol concentration, ethanol yield, and volumetric ethanol productivity at pH 5.5 and 6.0 provided the similar results (Table 4.2). The pH of Jerusalem artichoke juice that we used in this experiment was 5.6. Therefore, pH 5.5 was selected for further studies due to economical and practical reasons. The optimal pH range of medium for yeast growth can vary from pH 4.0 to 6.0, depending on growth conditions such as temperature, the presence of oxygen, and yeast strain (Narendranath and Power, 2005).

Table 4.2 Ethanol production by *K. marxianus* DBKKU Y-102 in Jerusalem artichoke juice supplemented with 230 g/l total sugar with various pHs at 37 and 40°C

pH	At 37 °C			At 40 °C		
	***P* (g/l)**	***Yps* (g/g)**	***Qp* (g/l.h)**	***P* (g/l)**	***Yps* (g/g)**	***Qp* (g/l.h)**
4.0	75.47±0.33[a] (36 h)	0.42±0.01[a]	2.09±0.01[a]	76.11±0.45[a] (48 h)	0.42±0.01[a]	1.59±0.01[a]
4.5	82.49±0.56[b] (36 h)	0.40±0.01[a]	2.29±0.02[b]	83.37±0.39[b] (48 h)	0.47±0.01[b]	1.74±0.00[b]
5.0	86.49±0.19[c] (36 h)	0.42±0.01[a]	2.40±0.02[c]	85.04±0.41[c] (48 h)	0.49±0.01[b]	1.78±0.01[c]
5.5	90.58±0.25[d] (36 h)	0.42±0.01[a]	2.51±0.01[d]	85.41±0.47[c] (36 h)	0.41±0.01[a]	2.37±0.01[d]
6.0	90.79±0.25[d] (36 h)	0.42±0.00[a]	2.52±0.01[d]	85.41±0.09[c] (36 h)	0.43±0.00[a]	2.37±0.01[d]

P, ethanol concentration produced (g/l); *Qp,* volumetric ethanol productivity (g/l.h).

[a-d] Means followed by the same letter within a same column are not significantly different using Duncan's multiple range test at the level of 0.05.

The results were expressed as mean ±SD.

The ethanol fermentation by the strain DBKKU Y-102 in the Jerusalem artichoke juice medium containing 230, 250 and 270 g/l sugar concentrations revealed that increasing the sugar concentration resulted in an increase in the final ethanol concentration but only up to 270 g/l sugar concentration at both 37 and 40°C (Table 4.3). The highest ethanol concentration (93.47±0.40 g/l), ethanol yield (0.47±0.0 g/g) and volumetric ethanol productivity (2.60±0.01 g/l.h) were achieved in a medium containing 250 g/l total sugar after 36 h of fermentation at 37°C. At 40°C, the highest ethanol concentration and volumetric ethanol productivity of 84.70±0.40 g/l and 2.36±0.01 g/l.h, respectively, were achieved at the same level of total sugar. Increasing the sugar concentrations resulted in a decrease in ethanol production. This might be attributed to the high osmotic pressure leading to cell disruption as described by Grubb and Mawson (1993). Based on the highest ethanol concentration obtained in

this study, the Jerusalem artichoke juice medium containing 250 g/l total sugar was chosen for next experiments.

Table 4.3 Ethanol production by *K. marxianus* DBKKU Y-102 in Jerusalem artichoke juice supplemented with various initial total sugars with pH 5.5 at 37 and 40°C

Total sugar (g/l)	At 37 °C			At 40 °C		
	P **(g/l)**	***Yps*** **(g/g)**	***Qp*** **(g/l.h)**	***P*** **(g/l)**	***Yps*** **(g/g)**	***Qp*** **(g/l.h)**
230	90.58±0.25[a] (36 h)	0.42±0.01[a]	2.51±0.01[a]	85.41±0.47[a] (36 h)	0.41±0.01[a]	2.37±0.01[a]
250	93.47±0.40[b] (36 h)	0.47±0.00[b]	2.60±0.01[b]	84.70±0.40[a] (36 h)	0.40±0.00[a]	2.36±0.01[a]
270	64.99±0.21[c] (48 h)	0.42±0.02[a]	1.36±0.00[c]	64.31±0.56[b] (36 h)	0.49±0.02[b]	1.79±0.02[b]

P, ethanol concentration produced (g/l); *Qp,* volumetric ethanol productivity (g/l.h).

[a-c] Means followed by the same letter within a same column are not significantly different using Duncan's multiple range test at the level of 0.05.

The results were expressed as mean ±SD.

The effect of initial cell concentrations of *K. marxianus* at 1×10^6, 1×10^7 and 1×10^8 cells/ml on ethanol production was determined in a Jerusalem artichoke juice medium containing 250 g/l sugar concentration and adjusted pH to 5.5. As showed in Table 4.4, the ethanol concentration increased when initial cell concentration increased. At 37°C, the maximum ethanol concentration (98.72±0.27 g/l), ethanol yield (0.46±0.00 g/g) and volumetric ethanol productivity (2.74±0.01 g/l.h) were achieved when the fermentation was carried out using 1×10^8 cells/ml. At 40°C, the maximum ethanol concentration (93.81±0.45 g/l), ethanol yield (0.43±0.03 g/g) and volumetric ethanol productivity (2.61±0.01 g/l.h) were achieved with an initial cell concentration of 1×10^8 cells/ml. These results are in good agreement with those reported by Thuesombat et al. (2007) who reported the optimal

conditions for ethanol production from Jerusalem artichoke juice after acid hydrolysis by *Saccharomyces cerevisiae,* i.e., pH 5.5, 250 g/l initial sugar concentration, 1×10^8 cells/ml initial yeast cell. Thanonkeo et al. (2011) also showed that the maximum ethanol concentration (95.9 g/l) with 98% of theoretical ethanol yield was obtained from ethanol fermentation in an acid hydrolysis of Jerusalem artichoke juice containing 250 g/l total sugar, pH 5.0 and inoculation size at 10% by *Zymomonas mobilis* TISTR548. The conventional techniques for the production of ethanol from Jerusalem artichoke tubers and juice consist of the acid or enzymatic hydrolysis of inulin, followed by fermentation of the resulting hydrolysates into ethanol (Onsoy et al., 2007; Thuesombat et al., 2007). These processes, however, have some disadvantages including by-product formation, product inhibition during hydrolysis, and subsequently, high production cost. In this study, thermotolerant yeast, *K. marxianus* DBKKU Y-102 exhibited high potential for ethanol production at high temperature from Jerusalem artichoke juice without acidic or enzymatic pre-treatment prior to fermentation.

Table 4.4 Ethanol production by *K. marxianus* DBKKU Y-102 in Jerusalem artichoke juice supplemented with 250 g/l total sugar and pH 5.5 with various initial cell concentrations at 37 and 40°C

Cell concentration (cells/ml)	At 37 °C			At 40 °C		
	***P* (g/l)**	***Yps* (g/g)**	***Qp* (g/l.h)**	***P* (g/l)**	***Yps* (g/g)**	***Qp* (g/l.h)**
1×10^6	93.47±0.40[a] (36 h)	0.46±0.00[a]	2.60±0.01[a]	84.70±0.40[a] (36 h)	0.40±0.01[a]	2.35±0.01[a]
1×10^7	94.73±0.01[b] (36 h)	0.45±0.03[a]	2.66±0.01[b]	89.54±0.17[b] (36 h)	0.47±0.00[b]	2.49±0.00[b]
1×10^8	98.72±0.27[c] (36 h)	0.46±0.00[a]	2.74±0.01[c]	93.81±0.45[c] (36 h)	0.43±0.03[c]	2.61±0.01[c]

P, ethanol concentration produced (g/l); *Qp,* volumetric ethanol productivity (g/l.h).

[a-c] Means followed by the same letter within a same column are not significantly different using Duncan's multiple range test at the level of 0.05.

The results were expressed as mean ±SD.

The effect of different nitrogen sources at various concentrations on ethanol fermentation by *K. marxianus* DBKKU Y-102 was investigated in a Jerusalem artichoke juice medium containing 250 g/l total sugar, adjusted pH to 5.5 and using initial cell concentration of 1×10^8 cells/ml. At 37°C, the highest ethanol concentration and ethanol productivity of 104.83±0.53 g/l and 4.37±0.02 g/l.h, respectively, were achieved from the medium supplemented with 0.5 g/l diammonium phosphate as a nitrogen source (Table 4.5).

Table 4.5 Ethanol production by *K. marxianus* DBKKU Y-102 in Jerusalem artichoke juice supplemented with 250 g/l total sugar, initial cell concentration of 1×10^8 cells/ml and pH 5.5 with various nitrogen sources at different concentrations at 37°C

Nitrogen source	Nitrogen concentration (g/l)	Ethanol parameter			Time (h)
		P **(g/l)**	***Yps*** **(g/g)**	***Qp*** **(g/l.h)**	
Control	0	95.39±0.26[a]	0.46±0.01[a,b]	3.97±0.01[a]	24
Diammonium phosphate	0.25	99.96±0.30[b]	0.47±0.01[b]	4.17±0.01[b]	24
	0.50	104.83±0.53[c]	0.47±0.00[b]	4.37±0.02[c]	24
	0.75	100.55±0.35[b]	0.45±0.01[a,b,c]	2.79±0.01[d]	36
	1.00	102.60±0.42[d]	0.45±0.00[a,b,c]	2.85±0.01[e]	36
Ammonium sulfate	0.25	100.64±0.33[b,e]	0.44±0.01[a,c]	2.80±0.01[d]	36
	0.50	101.46±0.10[e,f]	0.47±0.01[a,b]	2.82±0.00[e]	36
	0.75	102.24±0.28[d,f]	0.46±0.01[a,b,c]	2.84±0.01[e]	36
	1.00	100.07±0.42[b]	0.44±0.00[c]	2.78±0.01[d]	36
Yeast extract	6.00	102.00±0.42[d,f]	0.44±0.01[c]	4.25±0.02[f]	24
	9.00	76.38±0.45[g]	0.33±0.01[d]	3.18±0.02[g]	24
	12.00	80.14±0.48[h]	0.35±0.01[d,f]	3.34±0.02[i]	24
Corn steep liquor	20.00	81.92±0.52[i]	0.39±0.01[e]	3.41±0.02[i]	24
	30.00	80.46±0.25[h]	0.37±0.01[f,g]	3.35±0.01[g]	24
	40.00	81.50±0.12[i]	0.37±0.00[e,g]	3.40±0.01[i]	24

P, ethanol concentration produced (g/l); *Qp,* volumetric ethanol productivity (g/l.h).

[a-i] Means followed by the same letter within a same column are not significantly different using Duncan's multiple range test at the level of 0.05.

The results were expressed as mean ±SD.

At 40°C (Table 4.6), the ethanol concentrations produced by *K. marxianus* DBKKU Y-102 were in the range of 76.06-97.46 g/l. The highest volumetric ethanol productivity derived in this study was 3.98 g/l.h when the medium was supplemented with 0.5 g/l diammonium phosphate. It should be noted from this finding that ethanol concentration produced at 40°C was lower than that at 37°C. Since 0.5 g/l diammonium phosphate tends to be a good nitrogen source for ethanol production, therefore it was selected for further experiment. This is in contrast with Limtong et al. (2007), who reported the best nitrogen source for ethanol production from sugar cane juice by *K. marxianus* DMKU 3-1042 as 0.05% ammonium sulfate. Nuanpeng et al. (2011) reported that the maximum ethanol production efficiency from sweet sorghum juice by *S. cerevisiae* NP 01 was obtained when 9 g/l of yeast extract was supplemented to the medium. Pereira et al. (2010) reported the effect of corn steep liquor (CSL) on ethanol production from glucose medium by *S. cerevisiae*. The authors described that CSL is effective in significantly improving the kinetics of very high gravity (VHG) fermentations, permitting the reaction to reach its highest final ethanol titres and productivities. From these finding, we proposed that the optimal nitrogen source for ethanol production was depended on raw materials and microorganisms used in the process. Thomas and Ingledew (1990) reported that not all amino acids exhibit the same effect in promoting the growth of yeast. For example, glycine is readily taken up by yeast but it inhibits growth and ethanol fermentation. This inhibition may be resulted from the inability of the yeast to dispose of the two-carbon skeleton (glyoxylate) derived from glycine. It is known that some basic amino acids such as lysine and arginine inhibit growth and cell division in yeast (Cooper et al., 1979; Sumrada et al., 1978 cited in Thomas and Ingledew (1990).

Table 4.6 Ethanol production by *K. marxianus* DBKKU Y-102 in Jerusalem artichoke juice supplemented with 250 g/l total sugar, initial cell concentration of 1×10^8 cells/ml and pH 5.5 with various nitrogen sources at different concentrations at 40°C

Nitrogen source	Nitrogen concentration (g/l)	Ethanol parameter			Time (h)
		P **(g/l)**	***Yps*** **(g/g)**	***Qp*** **(g/l.h)**	
Control	0	91.78±0.11[a]	0.46±0.00[a]	3.82±0.00[a]	24
Diammonium phosphate	0.25	92.88±0.20[b]	0.43±0.01[b,c]	3.87±0.01[b]	24
	0.50	95.45±0.01[c]	0.44±0.01[b,c,d]	3.98±0.00[c]	24
	0.75	93.20±0.12[b]	0.43±0.02[b,c]	3.88±0.01[b]	24
	1.00	90.92±0.06[d]	0.43±0.00[b]	3.79±0.00[d]	24
Ammonium sulfate	0.25	97.46±0.05[e]	0.45±0.01[a,c,d]	2.71±0.00[e]	36
	0.50	97.11±0.54[e]	0.46±0.01[a,d]	2.70±0.01[e]	36
	0.75	94.07±0.24[f]	0.44±0.01[a,b,c,d]	2.61±0.01[f]	36
	1.00	92.92±0.16[b]	0.45±0.01[a,d]	2.58±0.00[g]	36
Yeast extract	6.00	90.87±0.49[d]	0.39±0.01[e]	3.79±0.02[d]	24
	9.00	76.06±0.23[g]	0.33±0.01[f]	3.17±0.01[h]	24
	12.00	77.21±0.16[h]	0.34±0.01[f]	3.22±0.01[i]	24
Corn steep liquor	20.00	79.53±0.23[i]	0.37±0.02[e,g]	3.31±0.01[j]	24
	30.00	79.94±0.47[i]	0.37±0.01[g]	3.33±0.02[j]	24
	40.00	79.84±0.21[i]	0.36±0.01[g]	3.33±0.01[j]	24

P, ethanol concentration produced (g/l); *Qp,* volumetric ethanol productivity (g/l.h).

[a-j] Means followed by the same letter within a same column are not significantly different using Duncan's multiple range test at the level of 0.05.

The results were expressed as mean ±SD.

Table 4.7 showed the effect of magnesium sulfate ($MgSO_4\cdot7H_2O$) on ethanol production was studied at the range of 0-2.0 g/l in a Jerusalem artichoke juice medium containing the sugar concentration of 250 g/l, using initial cell concentration of 1×10^8 cells/ml, 0.5 g/l diammonium phosphate, and adjusted the pH of medium to 5.5. At 37°C, there was not different in ethanol production, ethanol yield and ethanol productivity between media with and without additional of magnesium sulfate. At 40°C (Table 4.8), the highest ethanol concentration and ethanol productivity of

87.13±0.62 g/l and 3.63±0.03 g/l.h, respectively, were achieved in a medium without magnesium supplementation (Table 4.8). Magnesium is an important divalent cation essential for the metabolic processes and physiological functions, including cell growth, cell division and enzyme activity in yeast (Walker, 1994). In fermentation pathways, magnesium is required as a crucial cofactor in a variety of enzymes, including glucokinase, glucose-6-phosphate dehydrogenase, phosphoglycerate kinase and enolase. Magnesium levels are typically maintained at millimolar intracellular concentrations and would be one of the limiting factors under stress conditions. Specifically, magnesium plays a crucial role in the cellular protection resulting in reduction in cell mortality, prevention of cell-surface damage and repression of stress protein biosynthesis (Birch and Walker, 2000; Thanonkeo et al., 2007).

Table 4.7 Ethanol production by *K. marxianus* DBKKU Y-102 in Jerusalem artichoke juice supplemented with 250 g/l total sugar, initial cell concentration of 1×10^8 cells/ml, 0.5 g/l diammonium phosphate, and pH 5.5 with magnesium sulfate at various concentrations at 37°C

Magnesium sulfate concentration (g/l)	Ethanol parameter			Time (h)
	P **(g/l)**	***Yps*** **(g/g)**	***Qp*** **(g/l.h)**	
0	95.15±0.04[a]	0.45±0.00[a]	3.96±0.00[a]	24
0.50	95.20±0.30[a]	0.46±0.00[a]	3.97±0.01[a]	24
1.00	95.28±0.35[a]	0.46±0.00[a]	3.97±0.01[a]	24
1.50	94.97±0.23[a]	0.47±0.00[a]	3.96±0.01[a]	24
2.00	95.21±0.30[a]	0.46±0.00[a]	3.97±0.01[a]	24

P, ethanol concentration produced (g/l); *Qp,* volumetric ethanol productivity (g/l.h).

[a] Means followed by the same letter within a same column are not significantly different using Duncan's multiple range test at the level of 0.05.

The results were expressed as mean ±SD.

Table 4.8 Ethanol production by *K. marxianus* DBKKU Y-102 in Jerusalem artichoke juice supplemented with 250 g/l total sugar, initial cell concentration of 1×10^8 cells/ml, 0.5 g/l diammonium phosphate, and pH 5.5 with magnesium sulfate at various concentrations at 40°C

Magnesium sulfate concentration (g/l)	Ethanol parameter			Time (h)
	P **(g/l)**	***Yps*** **(g/g)**	***Qp*** **(g/l.h)**	
0	87.13±0.62[a]	0.45±0.01[a]	3.63±0.03[a]	24
0.50	84.78±0.27[b]	0.43±0.00[a]	3.53±0.01[b,c]	24
1.00	83.62±0.61[c]	0.44±0.00[a]	3.48±0.03[c,d]	24
1.50	83.32±0.15[c]	0.43±0.01[a]	3.47±0.01[d]	24
2.00	85.42±0.33[b]	0.43±0.00[a]	3.56±0.01[b]	24

P, ethanol concentration produced (g/l); *Qp,* volumetric ethanol productivity (g/l.h).

[a-d] Means followed by the same letter within a same column are not significantly different using Duncan's multiple range test at the level of 0.05.

The results were expressed as mean ±SD.

4.3.4 The batch ethanol fermentation by *K. marxianus* DBKKU Y-102 in a jar fermenter

The ethanol production in a 2L fermenter with a final working volume of 1.2L using a medium containing 250 g/l sugar concentration, cell concentration of 1×10^8 cells/ml, 0.5 g/l diammonium phosphate, pH of 5.5 and carried out at 37ºC was investigated. As showed in this study, the maximum ethanol concentration of 94.31±0.13 g/l, ethanol yield of 0.47±0.01 g ethanol/g sugar utilized and ethanol productivity of 2.62±0.00 g/l.h were achieved, with the theoretical ethanol yield of 91.19%. The viable cell count determined by methylene blue staining rapidly decrease after 24 h of fermentation and no viable cell could be detected at 48 h. This might be attributed to the high concentration of ethanol in the culture broth resulted in reduction of cell viability and inhibition of the cell growth (Ingram and Buttke, 1984; Alexander and Chapentier, 1998), which limits ethanol concentration in the broth to no more than 13% (v/v) for most ethanol production plants (Bai et al., 2004).

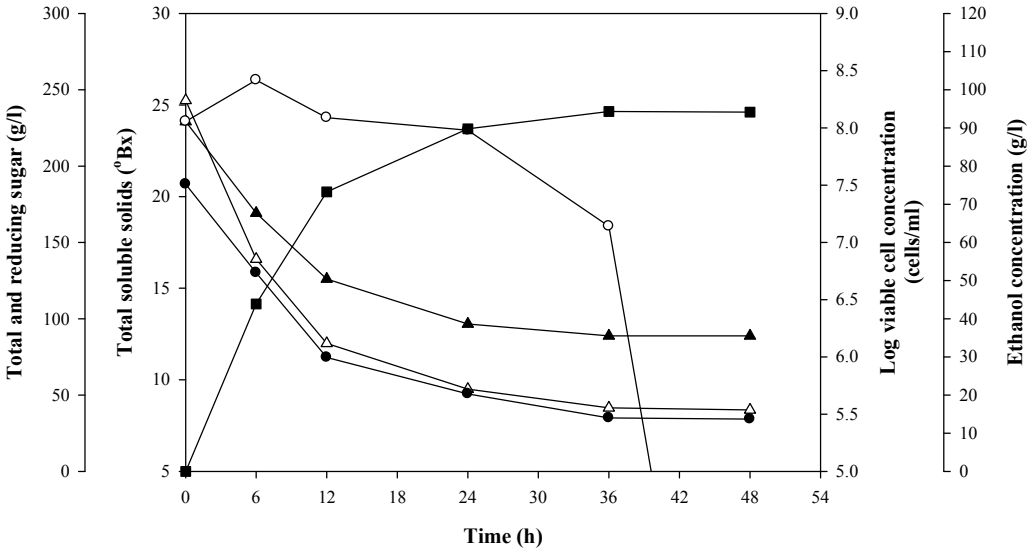

Figure 4.3 Ethanol production (■), total sugar (△), reducing sugar (●), log viable cell (○) and total soluble solids (ºBx) (▲) by *K. marxianus* DBKKU Y-102 in a Jerusalem artichoke juice medium supplemented with 250 g/l total sugar, 1×10^8 cells/ml initial cell number, 0.5 g/l diammonium phosphate and adjusted pH to 5.5 at 37°C in a 2L fermenter

Ethanol productions from different materials depend on many factors such as initial pH of the medium, initial cell concentration and strain of the microorganism use for fermentation. Table 4.9 showed the ethanol production data from various substrates using the different strains of *K. marxianus.* The highest volumetric ethanol productivity was achieved when the fermentation was performed by using *K. marxianus* DBKKU Y-102. This strain is well known for thermotolerance and inulin utilization, it shows potential in ethanol production from Jerusalem artichoke and the optimum ethanol fermentation temperature ranging from 30-40ºC (Hu et al., 2012; S. Kim and C.H. Kim, 2013; Margaritis and Bajpai, 1983). The highest ethanol concentration achieved in this study was 104.83±0.53 g/l when using a medium containing 250 g/l sugar concentration, cell concentration of 1×10^8 cells/ml, 0.5 g/l diammonium phosphate, pH of 5.5 and carried out at 37ºC in flask scale. The amount of ethanol achieved in this study was higher than that reported by Hu et al. (2012), although there are different in the initial substrate concentrations and the temperatures of ethanol fermentation.

Table 4.9 The ethanol production profiles from various substrates using the different strains of *K. marxianus*

Substrate	*P* (g/l)	*Qp* (g/l.h)	Temperature (°C)	Microbial strains	References
Jerusalem artichoke tubers (200 g/l)	60.9	1.02	35	*K. marxianus* ATCC8554	Yuan et al., 2007
Glucose (150g/l)	69	1.44	40	*K. marxianus* GX-UN120	Pang et al., 2010
Sugar beet thick juice diluted with whey and water (200 g/l)	102	1.42	30	*K. marxianus* KD-15	Oda et al., 2010
Crude whey (lactose 35 g/l)	2.10	0.18	34	*K. marxianus* MTCC 1288	Zafar and Owais, 2006
Lactose (44 g/l)	22	1.47	33	*K. marxianus* var. *marxianus* CBS 397	Sansonetti et al., 2011
Jerusalem artichoke flour (200 g/l)	73.6	1.53	40	*K. marxianus* PT-1	Hu et al., 2012
Jerusalem artichoke (10% (w/v) stalk and 8% (w/v) dried tuber powder)	45.3	1.51	30	*K. marxianus* CBS1555 (KCTC7001)	S. Kim and C.H. Kim, 2013
Jerusalem artichoke juice (250 g/l)	104.83	4.37	37	*K. marxianus* DBKKU Y-102	This study

P, ethanol concentration produced (g/l); *Qp,* volumetric ethanol productivity (g/l.h).

4.4 Conclusion

The results of this study demonstrated that the *K. marxianus* DBKKU Y-102 was the most effective strain capable of producing highest quantity of ethanol at elevated temperatures when Jerusalem artichoke juice was used as a raw material. The highest ethanol concentration of 104.83±0.53 g/l was achieved in a Jerusalem artichoke juice medium containing 250 g/l total sugar, 1×10^8 cells/ml initial cell number, 0.5 g/l diammonium phosphate and adjusted pH to 5.5 without magnesium sulfate supplementation at 37°C. The batch ethanol fermentation was conducted in a 2L jar fermenter under the optimal condition with an agitation speed of 100 rpm. *K. marxianus* DBKKU Y-102 yield the final ethanol concentration of 94.31±0.13 g/l, a productivity of 2.62±0.00 g/l.h, and 91.19% of the theoretical ethanol yield. These results suggested that the thermotolerant yeast, *K. marxianus* DBKKU Y-102, has high potential for ethanol production at high temperature from Jerusalem artichoke juice without pre-treatment.

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