ADAPTATION OF COMMERCIAL YEAST *SACCHAROMYCES CEREVISIAE* SC90 TO TOLERATE INHIBITORS GENERATED DURING CASSAVA PULP HYDROLYSIS

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Abstract

The pretreatment step of cellulosic ethanol production generates inhibitory compounds that affect the performance of fermenting microorganisms, and subsequently causes reductions in ethanol yield and productivity. Several researches in ethanol production from second generation feedstocks have therefore focused on the detoxification of inhibitors to increase the efficiency of the ethanol production. The adaptation of the microorganisms to inhibitors presented in raw material hydrolysate is one of the biotechnology routes to improve ethanol yield without any detoxification treatments. The purpose of this work was to adapt *Saccharomyces cerevisiae* SC90 to improve its ability to ferment toxic cassava pulp hydrolysate pretreated with diluted acid (1 N HCl). The initial total sugar of cassava pulp hydrolysate was 49.79 ± 0.76 g/l. The 20, 40, 60, 80, and 100% (v/v) of hydrolysate were prepared from the initial cassava pulp hydrolysate for the adaptation procedure. The total sugar concentration in all the diluted hydrolysate was adjusted to 50 g/l using glucose and supplemented with 1 g/l yeast extract, 2 g/l $(NH_4)_2SO_4$, 0.5 g/l KH_2PO_4, and 1 g/l $MgSO_4.7H_2O$ and the pH was adjusted to 5.5. The yeast cells were subcultured twice in the same hydrolysate dilution similar numbers of initial cells were transferred to a more concentrated hydrolysate. Adapted yeast cells from 100% (v/v) of hydrolysate supplemented with nutrient sources and non-adapted cells (the control) were investigated. The growth and fermentation potentials were compared. The adapted cells showed an improvement in sugar utilization and cell viability compared with those of the non-adapted cells. Consequently, the ethanol yield of the adapted cells was improved. These results indicated that the adaptation of the yeast cells to overcome the inhibitory effects of the lignocellulosic hydrolysate could improve the productivity and ethanol yield.

Keywords: adaptation, cassava pulp hydrolysis, inhibitors, *Saccharomyces cerevisiae*, tolerate

Introduction

The rapid increase in energy demand, excessive consumption of fossil fuels, and the limitation of global crude oil reserves need to be confronted. The utilization of fossil fuels

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has resulted in the negative impact on the environment (Sánchez and Cardona, 2008). Alternative energetic resources have therefore been considered. Bioethanol, a clean and renewable biofuel, is thought to be one of the best alternatives (Zaldivar *et al*., 2001). With regard to food security and sustainability, current research on bioethanol production has been remarkably focused on non-food lignocellulosic waste products as an environmentally-friendly potentially inexpensive, and more abundant alternative feedstock to fossil fuels (Wyman and Goodman, 1993)). Lignocellulosic biomass consists of 3 major constituents: cellulose, hemicelluloses, and lignin. Hemicelluloses are comprised of long chains of pentose sugars. Cellulose is composes composed of long chain glucose molecules and it is encapsulated by lignin. Therefore, these cellulosic materials are more difficult to hydrolyse than starch (Demirbaş, 2005). Cassava pulp represents one of the major lignocellulosic materials to be considered in most tropical countries on account of the high amount of starch on a dry weight basis (approximately 50-70%) but low cellulose fiber (approximately 20-30%) (Pandey *et al*., 2000). It is readily available at the cassava starch site as a fibrous by-product (Kosugi *et al*., 2009). This underused biomass has been evaluated for producing other value added bioproducts as well as biofuels.

Due to the structural characteristics of the lignocellulosic materials, a pretreatment step is crucial for obtaining potentially fermentable sugars in the hydrolysis step. It aims to break down complex structures to be a simple molecule of pentoses (xylose and arabinose) and hexoses (glucose, galactose, and mannose) and then ethanologenic microorganisms, usually yeast, can convert these sugars into ethanol (Leiper *et al*., 2006). The widely used pretreatment methods are steam explosion and acid hydrolysis. However, these methods result in the generation of toxic compounds that have deleterious effects on yeast cell growth and fermentation efficiency (Martín *et al*., 2006; Tomás-Pejo *et al*., 2010). Recent works have shown that the removal or detoxification of the inhibitors' toxicity including phenols, 5-hydroxy methylfurfural (5-HMF), furfural, and organic acids (Kahr *et al*., 2011) prior to its use as a fermentation medium improved ethanol productivity and yield (Martín *et al*., 2006). Nevertheless, the process is considered to limit the requirements for detoxification to a minimum for economic reasons (von Sivers *et al*., 1994) and the sugar losses may be significant according to the detoxification method used (Mussatto and Roberto, 2004). Microbial adaptation to inhibitor tolerance is therefore an alternative choice to overcome this problem (Chandel *et al*., 2011). Landaeta *et al*. (2013) minimized the negative effects of chemical inhibiters found in lignocellulosic hydrolysates such as acetic acid, furfural, hydroxyl methyl furfural, vanillin, syringaldehyde, and hydroxyl benzoic acid by acclimatization of a flocculent strain of *Saccharomyces cerevisiae* (NRRL Y-265) to a sequentially increased concentration of inhibitory compounds. By this strategy a yeast strain with an increased ethanol volumetric productivity and growth rate over the parental strain was obtained. The adaptation of *Pichia stipitis* NRRL Y-7124 to wheat straw hydrolysates showed an improved ethanol yield (Nigam, 2001). In addition, *P. stipitis* employed on a steam-stripped hardwood hydrolysate improved ethanol production with more than 90% xylose utilization compared with steam-unstripped hardwood hydrolysate (Parekh *et al*., 1987). Furthermore, adaptation of *Candida guilliermondii* employed on rice straw hemicellulose hydrolysate for xylitol production showed an effective and inexpensive method to alleviate the inhibitory effect of toxic compounds on the xylose to xylitol bioconversion (Silva and Roberto, 2001).

In this current study, the adaptation of the commercial yeast strain *S. cerevisiae* SC90 to inhibitory compounds generated during the pretreatment process was performed using sequential adaptation in order to improve the laboratory scale of ethanol production from cassava pulp hydrolysis. Experiments were conducted to compare the

adapted cells from 100% (v/v) of hydrolysate supplemented with nutrients and the non-adapted cells (the control) in growth and fermentation potentials.

Materials and Methods

Strain, Medium, and Culture Conditions

The commercial yeast strain, *S. cerevisiae* SC90, obtained from the Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok has already been used for ethanol production on an industrial scale by several alcoholic beverage factories in Thailand. The strain was maintained and grown on yeast extract peptone dextrose (YPD) containing 20 g/l neutralised bacteriological peptone, 10 g/l yeast extract, and 20 g/l glucose. Agar 20g/l was added when solid media were required. A starter culture was obtained by inoculating the yeast from the YPD slope into 10 ml of the YPD medium. This was incubated in an orbital shaker at 120 rpm and 30°C for 48 h. An aliquot of 10 ml was then transferred to a 250 ml baffle flask containing 100 ml of fresh YPD and incubated as stated above.

Pretreatment of Cassava Pulp

Cassava pulp was obtained from S.C. Industry Co. Ltd., Chachengsao, Thailand. It was dried and milled before use. The cassava pulp for adaptation was prepared by pretreating 100 g of cassava pulp with 900 ml of diluted acid (1 N HCl) and a steam explosion at 132°C for 1 h. The 100% (v/v) of hydrolysate supplemented with 1 g/l yeast extract, 2 g/l $(NH4)_2SO_4$, 0.5 g/l KH_2PO_4, and 1 g/l $MgSO_4.7H_2O$ was the initial. The pH was adjusted to 5.5. The hydrolysates were diluted to 20, 40, 60, 80% (v/v) with distilled water and glucose was added to all diluted hydrolysates to adjust the total sugar concentration similar to that in 100% (v/v) of hydrolysate. The other nutrients were supplemented similar to the 100% hydrolysate (Tomás-Pejo *et al*., 2010). The media were sterilized at 121°C for 15 min.

Adaptation Procedure

The strain was pre-grown in YPD medium. Cells were incubated in an orbital shaker at 30°C and 120 rpm for 48 h. A direct adaptation method was modified from Tomás-Pejo *et al*. (2010). Five sequentially different media with an increasing inhibitor concentration were prepared (Figure 1). The medium was inoculated with *S. cerevisiae* SC90 to achieve an initial cell concentration of 3×10^7 cfu/ml. Yeast cells were subcultured twice after 48 h in the same hydrolysate concentration before a similar number of initial cells were transferred to a more concentrated hydrolysate (gradually increasing the concentration to 40%, 60%, 80%, and 100%). The adaptation procedure was carried out in a laboratory scale fermentation using a conical flask with an air lock on an orbital shaker at 30°C and 120 rpm. All sequential adaptations were performed in triplicate. Cell growth and viability were examined. The obtained strain (SC90-5) was cultivated on YPD agar plates supplemented

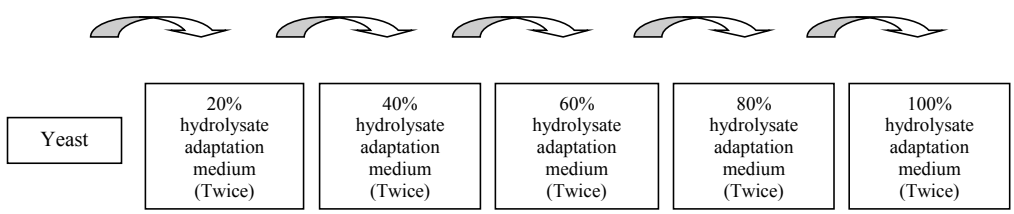

Figure 1. Adaptation procedure of *S. cerevisiae* SC90 on cassava pulp hydrolysate pretreated by diluted acid and steam explosion. Adaptation was carried out in a laboratory scale fermentation on an orbital shaker at 30°C and 120 rpm

with 1 g/l furfural and 4 g/l acetic acid for maintenance and further evaluation, as recommended by Landaeta *et al*. (2013).

The adapted cells and the non-adapted cells (the control) fermentations were carried out in 100% (v/v) of hydrolysate with the same conditions as the adaptation procedure. Samples were collected to investigate growth, total sugar utilization, and ethanol contents. The data set was calculated for presentation in terms of the kinetic parameters.

Analysis of Fermentation Substrates and Products

Viable cell counts: Cell suspensions were diluted to an appropriate concentration and density was measured using a counting chamber and a standard light microscope. Viability was determined by mixing a volume of cell suspension with an equal volume of methylene blue solution (methylene blue dissolved in 2% (w/v) sodium citrate solution to a final concentration of 0.01% (w/v)). Unstained cells are assumed to be viable. The viability of the sample was expressed as a percentage. At least 500 cells were enumerated and calculated for the viability (Smart *et al*., 1999).

Glucose utilization: Total sugar content was assessed using the phenol sulphuric method (Dubois *et al*., 1956). The fermentation data set was calculated to the glucose utilization rate with the unit of glucose consumed in grams per hour.

Ethanol production: Ethanol concentration was determined by gas chromatography with a gas chromatographer mass spectrometer (Shimadzu GCMS-QP2010 Ultra, Shimadzu Corp., Kyoto, Japan). Iso-propanal was used as the internal standard. Ethanol yields and percent theoretical yields were calculated using the following equations (Keating *et al*., 2006):

$$Y_{P/S} = [EtOH]_{max} \div [Sugar]_{ini} \quad (1)$$

$$Q = [EtOH]_{max} \div \text{Attenuation time} \quad (2)$$

$$Y_{\%T} = (Y_{P/S} \div 0.51) \times 100 \quad (3)$$

where $Y_{P/S}$ = ethanol yield (g/g), $[EtOH]_{max}$ = maximum ethanol concentration achieved during fermentation (g/l), $[Sugar]_{ini}$ = total initial sugar concentration at onset of fermentation (g/l), Q = volumetric productivity (g/l/h), Attenuation time = time taken to reach maximum ethanol, $Y_{\%T}$ = percent theoretical yield (%), and 0.51 = theoretical maximum ethanol yield per unit of hexose sugar from glycolytic fermentation (g/g).

Statistical Method

The mean and standard deviation of a data set was calculated using the AVERAGE and STDEV functions of Microsoft® Excel 2003. The presented results were expressed as the mean ± STDEV obtained from 3 independent experiments and error bars correspond to the STDEV. Statistical analysis of data was conducted by analysis of variance: single factor at 95% confidence limits.

Results and Discussion

Pretreatment Hydrolysis of Cassava Pulp Using Diluted Acid and Steam Explosion

Cassava pulp, a solid by-product from the starch industry, is a promising and underused biomass that can be converted into valuable biofuels. Its dried 50-70% starch, 15.63% cellulose, 4.58% hemicellulose, and 2.83% lignin (Pandey *et al*., 2000; Rattanachomsri *et al*., 2009). Starch, cellulose, and hemicellulose can be converted to fermentable monomeric sugars in the pretreatment step. Pretreatment hydrolysate with1N HCl at 132°C for 1 h contained 49.79±0.76 g/l total sugars. It was used to prepare the adaptation media. The concentration of total sugars in each hydrolysate dilution was adjusted to be around 50 g/l before hydrolysate sterilization. Sugars are found in biofuel production including hexose (sucrose, glucose, fructose, maltose, and galactose) and pentose (arabinose and xylose) (Prasad *et al*., 2007). It was reported that hexose is the major sugar in the solid fraction of softwood, which was favorable for bioconversion to ethanol (Prasad *et al*., 2007).

Cell Growth in a Series of Concentration of Inhibitors

Successful adaptation of yeasts to rice straw hemicellulose and softwood prehydrolysates has been reported and also that adapted strains performed with higher resistance to inhibitors compared with the parental strain (Keller *et al*., 1998; Silva and Roberto, 2001). Nevertheless, adaptation of the commercial strain of *S. cerevisiae* SC90 to cassava pulp hydrolysate has not been reported previously.

Cell growth and viability under the sequential adaptation of each hydrolysate dilution was examined using methylene blue staining (Figure 2(a and b)). The data collected from the first run of each adaptation demonstrated that the maximum viable cell number was observed at 20% hydrolysate with $1.36 \pm 0.09 \times 10^8$ cfu/ml within 48 h compared with cell growth in normal YPD at $1.37 \pm 0.18 \times 10^8$ cfu/ml (data not shown). When a higher concentration of hydrolysate (80 and 100% hydrolysate) was used, yeast cells responded with an extended lag period of 4 h prior to cell growth. The growth rate of yeast cells confronted with higher inhibitor contents (40 and 60% hydrolysate) appeared to be lower with the final cell number of $5.31 \pm 0.2 \times 10^7$ to $5.33 \pm 0.24 \times 10^7$ cfu/ml. Moving to a higher concentration of inhibitors at 80 and 100% hydrolysate, cells have a longer lag period and the maximum cell number was $2.69 \pm 0.05 \times 10^7$ to $4.11 \pm 0.26 \times 10^7$ cfu/ml.

Thermochemical degradation of hemicellulose provides the majority of sugar monomers including xylose, mannose, galactose, and glucose which coincides with a number of inhibitory compounds that are toxic to the fermenting microorganism (Parawira and Tekere, 2011). Our results suggested that a concentration of hydrolysate exceeding 20% might contains a minimum amount of inhibitors that can cause injury to cells. The hydrolysate obtained in our research might be composted of furfural, 5-hydroxymethylfurfural (5-HMF), acetic acid, and formic acid which are generally found during dilute acid pretreatment. These toxic compounds affect overall cell physiology and often result in decreased viability, ethanol yield, and productivity (Palmqvist and Hahn-Hägerdal, 2000a; Chandel et al., 2011), and the breakdown of DNA (Endo *et al*., 2008). It was experimentally confirmed by Tian *et al*. (2009) that furfural and 5-HMF mainly affected the length of the lag phase during the initial part of the incubation process.

According to the current study, it was hard to improve cell growth in hydrolysate containing a high concentration of inhibitors. This yeast strain was not properly adapted to deal with the high number of inhibitors, which was also apparent from the relatively poor rate of cell growth in these hydrolysates. This is therefore a big challenge for further studies to enhance both cell density and viability.

Figure 2(b) presents the percentage viability of SC90 during sequential adaptation. The data collected from the first run of each adaptation showed that at 20% hydrolysate, viability declined from 100% to 97.80 ± 1.08%. After the end of the second run, the cells were then transferred to 40% hydrolysate. At this condition, the cell viability seemed to be maintained at 84-86% throughout the fermentation period. Moving the cells from 40% hydrolysate to 60% hydrolysate surprisingly did not caused more cell death, but higher cell viability was seen, even though, a smal amount of cell growth was found, as presented in Figure 2(a). This was probably because the cell adaptation process of *S. cerevisiae* SC90 could occur during the extended lag phase when cells were confronted with a higher concentration of inhibitors presented in the cassava pulp hydrolysate. Once a culture is able to tolerate the inhibitors, active cell growth and metabolism will recover (Tian *et al*., 2011). Acclimatization of the fermentative microorganisms to inhibitory compounds generated during the pretreatment process prior to fermentation of lignocellulose hydrolysates is able to enhance product yields (Parawira and Tekere, 2011).

Hydrolysate Fermentation Performance of Adapted Cells and Non-adapted Cells

The adaptation procedure of the commercial yeast *S. cerevisiae* SC90 to tolerate inhibitors generated during thermochemical hydrolysis of the cassava pulp was developed by sequential adaptation strategy. To evaluate this procedure, adapted cells from 5 series of adaptation and non-adapted cells were inoculated into 100% hydrolysate supplemented with a variety of nutrients and incubated at 120 rpm and 30°C for 72 h. Then growth and fermentation performance were compared.

Monitoring of Cell Growth

Viable cell counts and the viability of adapted cells and non-adapted cells (control) were examined (Figure 3). During the first 8 h of fermentation, there were small differences in the viable cell counts between the non-adapted and adapted cells. However, after 48 h, the cells grown in the 5 sequential adaptations demonstrated higher viable cell density than the non-adapted cells. The viable cell count of fermentation conducted with the adapted cells showed a maximum viable cell number at $4.35 \pm 0.11 \times 10^7$ cfu/ml at 42 h and then seemed to be stable until 72 h. However, the maximum viable cell count of the non-adapted cells appeared to be lower with a final cell number around $4.03 \pm 0.14 \times 10^7$ cfu/ml at 42 h and then it gradually declined to $3.57 \pm 0.12 \times 10^7$ cfu/ml at 72 h. A reduction in viability was observed according to the high content of fermentation inhibitors (Martín *et al.*, 2006), ethanol and CO_2 accumulation, and nutrient limitation (Gibson *et al.*, 2007). Our results suggested that adaptation led to an improvement of the evolved strain to be more tolerant to the toxic compounds present in the medium, as proved by better growth in the adapted cells. Liu (2009) has also shown that the adapted strain is able to withstand the inhibitor stress and that rapid cell growth after 42 h was observed, whereas the parental strain is unable to recover. The adapted strain displays an enriched genetic background with a significantly higher number of gene transcripts than the non-tolerant parental strain. In addition, the adapted strain might be able to reduce the toxic compounds by altering the chemical nature of the inhibitors in the hydrolysates (Martín *et al.*, 2006; Chandel *et al.*, 2011). For example, *S. cerevisiae* grown in anaerobic conditions is able to detoxify furans by reducing to its corresponding alcohol moieties (Palmqvist and Hahn-Hägerdal, 2000b;

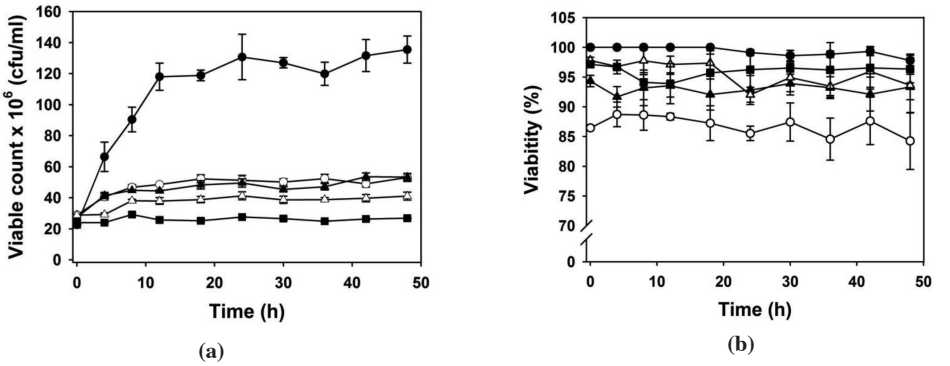

Figure 2. Viable cell count (A) and viability (B) of *S. cerevisiae* SC90 during 5 series of adaptation. The data collected from the first run of each adaptation is demonstrated. Yeast cells were subcultured twice at the same hydrolysate before transferring to a higher concentration of hydrolysate (20% hydrolysate, ●; 40% hydrolysate, ○; 60% hydrolysate, ▲; 80% hydrolysate, △; 100% hydrolysate, ■)

Mussatto and Roberto, 2004). *S. cerevisiae* partially metabolizes some of the phenolic compounds to its less toxic vinyl derivatives probably via phenylacrylic acid decarboxylase conversion of cinnamic, *p*-coumaric, and ferulic acids (Clausen *et al*., 1994; Larsson *et al*., 2000). Some *Saccharomyces* strains have the capability to transform 5-HMF to the less toxic 2,5-bis-hydroxymethyl-furan (Liu *et al*., 2004) and cells metabolize 5-HMF by increasing the expression of the ADH6 (alcohol dehydrogenase 6) gene (Petersson *et al*., 2006). In addition, yeast expresses furan reductase to enhance the fermentation rate (Larsson *et al*., 2001; Nilsson *et al*., 2005). However, in this current study the types and amounts of the inhibitors in the cassava pulp hydrolysate were not determined.

Sugar Utilization and Ethanol Production

According to the rate of total sugar utilization in terms of glucose (Figure 4(a)), the adapted cells show a faster rate of sugar utilization compared with the non-adapted cells. Even though there are many kinds of toxic inhibitors generated during pretreatment of hemicelluloses hydrolysate, Keating *et al.* (2006) reported that furfural and acetic acid had little independent effect upon sugar consumption rates. After 36 h, the residual glucose was 11.04 ± 0.18 g/l in the fermentation with the adapted cells, whereas at 48 h of the fermentation period, a higher amount of glucose at 15.73 ± 0.62 g/l had still not been consumed by the non-adapted cells. Apart from the effect of toxicification of the inhibitors on the sugar consumption rate, there still seemed to be residual sugars in the process regarding unfermentable sugars, including xylose and arabinose (Dwivedi *et al*., 2009) in which *S. cerevisiae* cannot metabolize. Figure 4(b) shows the ethanol content of the adapted and non-adapted cells determined by gas chromatography. Ethanol production by the adapted cells reached the highest content of 16.98 ± 1.13 g/l at 36 h. For the non-adapted cells, the maximum concentration of the ethanol produced was lower at 12.79 ± 1.04 g/l. It also took a longer time to reach the maximum ethanol concentration (48 h).

Kinetic Parameters of Adapted Cells and Non-adapted Cells on Ethanol Production

Hydrolysate from pretreatment of diluted acid and steam exploded cassava pulp was used in the fermentation of the parental and adapted cells to compare the kinetic parameters of the attenuation time, rate of sugar utilization, residual sugar, ethanol concentration, ethanol yield, volumetric productivity, as well as fermentation efficiency (Table 1).

The non-adapted cells presented a poor fermentation performance compared with the adapted cells. It probably was due to the

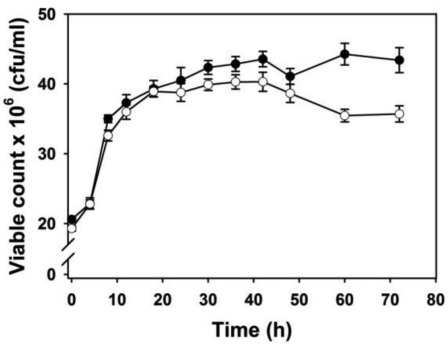

Figure 3. Viable cell count of *S. cerevisiae* SC90. Adapted cells (●) from 5 series of adaptation and non-adapted cells (○) were inoculated into 100% hydrolysate and incubated at 120 rpm and 30°C for 72 h

presence of biomass degradation by-products mainly furfural, acetic acid, and compounds from lignin and extractives (Clark and Mackie, 1984). The adapted cells significantly performed all kinetic parameters better than the non-adapted cells. This proved the success of the directed evolution with regard to improved sugar consumption rates. The higher amount of consumed glucose was reflected in higher fermentation efficiency. It was found that an improvement of 32.76, 20.00, 76.00, and 20.00% in ethanol content, ethanol yield, volumetric productivity, and fermentation efficiency, respectively, was achieved using the adapted cells. In addition, faster sugar utilization by the adapted cells suggested that the ability to tolerate and/or metabolize toxic inhibitors was enhanced (Keating *et al*., 2006; Martín *et al*., 2006; Tomás-Pejo *et al*., 2010). Our results can be supported by the findings of other researches on adaptation of fermenting yeast to lignocellulosic hydrolysate for improving ethanol production, as presented in Table 1. Using sequential adaptation, yeast presents higher inhibitor tolerance which results in a higher rate of sugar utilization and finally improvement in ethanol fermentation efficiency compared with the parental strain (non-adapted cells) (Martín *et al*., 2006; Tomás-Pejo *et al*., 2010; Landaeta *et al*., 2013). Interestingly, the adapted cells in this current research which passed 5 runs of a series of adaptation and were then inoculated in 100% hydrolysate demonstrated an improvement in sugar utilization with a lower residual glucose compared with the non-adapted cells grown in 100% hydrolysate. This indicated that more runs of adaptation gave more improvements, as presented in the work of Keating *et al*. (2006) that showed only a mild improvement in the ethanologenic capacity via cell recycling.

Conclusions

Adaptation of the commercial ethanologenic strain of *S. cerevisiae* SC90 to cassava pulp hydrolysate with increasing concentrations of inhibitors provided further improvement in the fermentative competence with respect to higher glucose utilization rates, higher ethanol contents, and higher productivities than the non-adapted cells, which should be beneficial to economization. The results clearly indicate the great potential in using adaptation as an effective strategy for improving yeast tolerance to the toxic compounds in cassava pulp hydrolysate.

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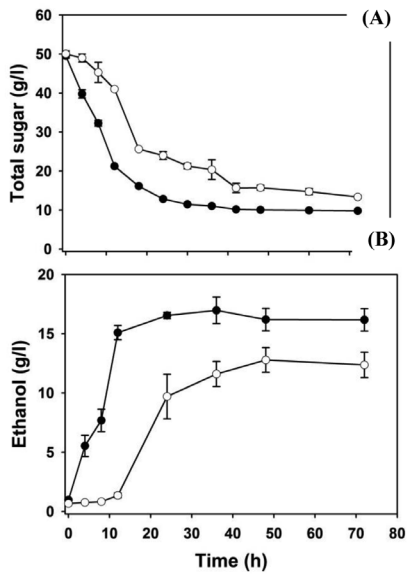

Figure 4. Changes in total sugars (A) and ethanol content (B) of *S. cerevisiae* SC90. Adapted cells (●) from five series of adaptation and non-adapted cells (○) were inoculated into 100% hydrolysate and incubated at 120 rpm and 30°C for 72 h

Table 1. Summary of kinetic parameters for *S. cerevisiae* SC90

Conditions	Attenuation time (h)	Rate of sugar utilization (g/l/h)	Residual sugar (g/l)	Ethanol concentration (g/l)	Ethanol yield ($Y_{P/S}$ g/g)	Volumetric productivity (Q g_P/l/h)	Fermentation efficiency ($Y_{\%T}$)
Non-adapted cells (the control)*	48	0.71 ± 0.02^a	15.73 ± 0.62a	12.79 ± 1.04^a	0.35 ± 0.02^a	0.25 ± 0.01^a	68.49 ± 4.68^a
Adapted cells* (Present work)	36	1.07 ± 0.02^b	11.04 ± 0.18b	16.98 ± 1.13b	0.42 ± 0.03^b	0.44 ± 0.02^b	82.19 ± 5.14^b
Non-adapted cells Adapted cells	98	-	-	-	0.48	0.48	93.93
(Landaeta *et al*., 2013)	75	-	-	-	0.48	0.53	93.93
Non-adapted cells Adapted cells	140	-	-	21.80	0.23	0.16	44.00
(Tomás-Pejo *et al*., 2010)	140	-	-	27.40	0.31	0.20	61.00
Non-adapted cells Adapted cells	36	-	-	-	0.38	0.23	74.36
(Martín *et al*., 2006)	24	-	-	-	0.38	0.51	74.36

Mean values in each column with different letters indicate significant differences ($p \leq 0.05$). All values are expressed as means ± standard deviation (n = 3). Attenuation time is time taken to reach maximum ethanol. Residue sugar is calculated at the attenuation time.
**refers to this current research.*

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