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ORIGINAL PAPER

Enhancement in xylose utilization using *Kluyveromyces marxianus* NIRE-K1 through evolutionary adaptation approach

Nilesh Kumar Sharma[1,2] · Shuvashish Behera[1] · Richa Arora[1,2] · Sachin Kumar[1]

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Abstract The evolutionary adaptation was carried out on the thermotolerant yeast *Kluyveromyces marxianus* NIRE-K1 at 45 °C up to 60 batches to enhance its xylose utilization capability. The adapted strain showed higher specific growth rate and 3-fold xylose uptake rate and short lag phase as compared to the native strain. During aerobic growth adapted yeast showed 2.81-fold higher xylose utilization than that of native. In anaerobic batch fermentation, adapted yeast utilized about 91 % of xylose in 72 h and produced 2.88 and 18.75 g l^{-1} of ethanol and xylitol, respectively, which were 5.11 and 5.71-fold higher than that of native. Ethanol yield, xylitol yield and specific sugar consumption rate obtained by the adapted cells were found to be 1.57, 1.65 and 4.84-fold higher than that of native yeast, respectively. Aforesaid results suggested that the evolutionary adaptation will be a very effective strategy in the near future for economic lignocellulosic ethanol production.

Keywords Lignocelluloses · *Kluyveromyces marxianus* · Xylose · Evolutionary adaptation · Ethanol · Xylitol

Introduction

Environmental and ecological imbalances are the two major concerns for the development of man, which can be fulfill by the replacement of conventional petroleum energy sources with renewable energy resources [1, 2]. For the last three decades, bioethanol produced from renewable feedstocks have received much attention due to their potential role to replace conventional fuels. However, the use of readily fermentable agricultural feedstocks including sugarcane juice and corn is less desirable for the production of bioethanol because of its negative connotation in the food versus fuel debate [3]. Currently, researchers and entrepreneurs are aiming at lignocellulosic biomass (LCB) which contains lignin, cellulose, and hemicelluloses, where, cellulose and hemicellulose undergo hydrolysis yielding fermentable sugars like glucose and xylose [2, 4–9]. Besides, xylose is the second most abundant sugar, making 5–35 % share of total dry LCB [10–12]. However, both pentose and hexose sugars must be utilized for economical production of lignocellulosic ethanol [13, 14].

The traditional yeast *Saccharomyces cerevisiae* is efficient to ferment glucose but unable to ferment xylose for ethanol production [15]. Xylose fermentation and subsequent ethanol production have been studied in several xylose fermenting yeasts, including *Scheffersomyces* (*Pichia*) *stipitis*, *Candida shehatae*, *Pachysolen tannophilus* and *Kluyveromyces marxianus* [12]. However, their ethanol productivities are very poor as compared to *S. cerevisiae* when glucose based substrates are used [16]. There are some other limitations including ethanol and sugar tolerance, undesired by-product formation, limited substrate specificity, redox imbalance and low ethanol productivity [17]. The majority of the studies have

N. K. Sharma and S. Behera equal contributors.

✉ Sachin Kumar
sachin.biotech@gmail.com

[1] Biochemical Conversion Division, Sardar Swaran Singh National Institute of Bio-Energy, Kapurthala, India

[2] I.K Gujral Punjab Technical University, Kapurthala, India

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conducted using metabolic engineering through a rational selection of genes to be manipulated for the development of novel pentose-fermenting strains with varying levels of success [18]. However, the mechanisms by which pentose-fermenting yeasts accomplish to increase the rate of ethanol production are still not fully explored. The simultaneous co-fermentation of hexose and pentose sugars also constitutes a major challenge in strain development [19].

Efforts have also been made to establish a xylose-utilizing pathway in *S. cerevisiae* by insertion of the genes encoding xylose reductase and xylitol dehydrogenase from *S. stipitis* and xylose isomerase from bacteria and fungus, resulted in poor ethanol production from xylose together with undesired metabolites. Major attention has been paid to *S. cerevisiae* and other mesophilic yeast strains including *S. stipitis* and *C. intermedia* for development of genetically modified model for ethanol production from lignocellulosic biomass [12, 20, 21]. However, mesophiles have limitation including fermentation and saccharification at low temperatures, energy requirements for mixing and product recovery, which increases cost of process [22, 23]. On the other hand, theromophilic and theromotolerent yeast strains have many advantages over mesophiles, i.e. higher saccharification and fermentation rate, broad substrate utilization range together with less energy requirement for mixing and product recovery, lower cost of pumping and steering, no cooling required and minimized contamination risk [5, 24]. *K. marxianus* carrying all aforesaid advantages and will be the better alternative to *S. cerevisiae* and *S. stipitis* for ethanol production process in near future [24–27].

Evolutionary engineering (evolutionary adaptation) is an important strategy to enhance strains with desired production traits [28, 29]. Various researchers have employed this strategy to generate improved xylose fermenting strains [30–35]. The potentially adaptive evolution has been elucidated by several researchers in improving the growth and fermentation process of microorganism, when xylose is used as sole source of carbon [29, 34]. The adaptation of fermenting microorganisms to inhibitory lignocellulosic hydrolysates is another possible tool for dealing with inhibitor problems [36–38]. Also adaptive evolution has been employed to the recombinant microbial strains to increase their fermentation capability [28, 31, 39]. However this strategy does not affects any genetic changes [40].

The present study was explored an improved xylose utilizing strain for lignocellulosic bioethanol production having improved xylose uptake rate in *K. marxianus* NIRE-K1 through evolutionary adaptation strategy. The kinetic parameters of adapted strain have also been reported and compared with native strain.

Materials and methods

Microorganism and media

Thermotolerant yeast *Kluyveromyces marxianus* NIRE-K1 (MTCC 5933) was used for evolutionary adaptation study has been reported for the utilization of glucose and xylose by Arora et al. [41]. The yeast culture was grown at 45 °C and maintained on yeast extract-peptone (YEP) medium [(g l^{-1}): yeast extract (Himedia, Mumbai, India), 10; peptone (Himedia, Mumbai, India), 20; glucose/xylose (Himedia, Mumbai, India), 20; phytagel (Sigma-Aldrich, USA), 15; pH, 5.5]. Yeast culture was maintained at −80 °C in stock with 30 % glycerol.

Growth condition

Growth profiling under aerobic condition was carried out in 500 ml Erlenmeyer flask in triplicate manner with working volume of 100 ml YEP medium containing 2 % (w/v) xylose/glucose. Growth was performed for 24 h at 45 °C and 150 rpm on an orbital shaker incubator for the cells of native and adapted, respectively. The samples were collected at interval of 2 h and analyzed for dry cell weight (DCW), xylose concentration and extracellular metabolites.

Evolutionary adaptation

Evolutionary adaptation in repetitive batch culture was carried out through sequential transfer to YEP medium containing 2 % xylose as carbon source. Adaptation was carried out in 100 ml of cotton plugged Erlenmeyer flask with 20 ml of medium. The initial batch was performed by culturing loopful cells from phytagel plate in Erlenmeyer flask and incubated for 24 h at 45 °C in an orbital shaker incubator (New Brunswick Innova 43/43R Shaker, Germany) at 150 rpm. Furthermore, 1 % inoculum was used for consecutive batch for subsequent growth. 1 ml sample was withdrawn after every 24 h for analysis of residual xylose, DCW and metabolites produced during the growth. The adaptation continued up to 60 batches until the adapted culture was able to utilize more than 80 % (w/v) xylose sugar.

Fermentation conditions

Fermentation analysis was carried out to determine the capability of adapted strain as compared to native strain. Inoculum for fermentation by native and adapted *K. marxianus* NIRE-K1 were prepared using YEPX medium with similar composition as that of growth medium except xylose concentration as 30 g l^{-1}. Anaerobic fermentation was carried out in 1000 ml screw-caped erlenmeyer flask

containing 200 ml YEPX medium at a temperature of 45 °C and pH 5.5 with agitation of 150 rpm on an orbital shaker incubator. The initial cell mass concentration was kept at 2 g l^{-1}.

Analytical methods

Samples withdrawn during growth and fermentation were centrifuged at 10,000 rpm for 15 min and collected samples were stored at 4 °C. Various metabolites in fermentation broth (glucose, xylose, ethanol, xylitol, glycerol and acetic acid) were analyzed using HPLC (Agilent Technologies) with HiPlex H column at 57 °C with 1 mM H_2SO_4 as the mobile phase at a flow rate of 0.7 ml min^{-1} and detected by Refractive Index Detector at 50 °C.

DCW was obtained by centrifuging 1 ml of sample in pre-dry weighted micro centrifuge tube using Eppendorf centrifuge 5430 R at 10,000 rpm for 15 min, followed by washing the pellet twice with deionized water and further drying in a vacuum oven at 80 °C to a constant weight. All the experiments were carried out in triplicate, and the given values are the mean values.

Kinetics

Fermentation kinetic parameters were calculated using the following formulae by Bailey and Ollis [42]:

$$\mu = \text{Standardized value for specific growth rate of } K.\,marxianus \text{ NIRE-K1} \quad (1)$$

$$Y_{X/S} = \frac{\text{Mass of biomass (yeast cell) formed}}{\text{Mass of substrate (xylose) consumed}} \quad (2)$$

$$Y_{P/S} = \frac{\text{Mass of product formed}}{\text{Mass of substrate (xylose) consumed}} \quad (3)$$

$$Q_S = \text{Substrate (xylose) uptake (g) per liter per hour} \quad (4)$$

$$Q_P = \text{Product formed (g) per liter per hour} \quad (5)$$

$$q_S = \frac{\text{Mass of substrate (xylose) consumed}}{\text{Mass of biomass (yeast cell) formed}}\mu \quad (6)$$

$$q_P = \frac{\text{Mass of product formed}}{\text{Mass of biomass (yeast cell) formed}}\mu \quad (7)$$

Results and discussion

The potential of the isolated native yeast strain *K. marxianus* NIRE-K1 for ethanol fermentation on glucose has been reported with an ethanol yield of 0.39 ± 0.03 g g^{-1} at 45 °C using salt medium [41]. However, the strain showed less growth with very slow sugar utilization, when grown on medium containing xylose as the sole carbon source. In this study, an assessment of the native and adapted strain was done in terms of xylose utilization, DCW, sugar uptake rates, ethanol and xylitol production by using YEPX medium during growth and batch fermentations.

Adaptation of *K. marxianus* NIRE-K1

An evolutionary adaptation was conducted to evaluate reproducibility of strategy by repeated batch growth of *K. marxianus* NIRE-K1 in YEP medium containing 20 g l^{-1} xylose for 24 h at 45 °C and monitored improvement in growth and sugar assimilation after each batch. Up to first seven batches very little improvement was observed in xylose utilization i.e. 18.46 only (Fig. 1). However, a noticeable improvement was observed after completion of around 10 batches. Subsequently, after 50 batches, *K. marxianus* NIRE-K1 was able to utilize 17.40 g l^{-1} of xylose out of 20 g l^{-1} of initial xylose, which shows 87 % of sugar utilization with the formation of 4.1 g l^{-1} biomass. Adaptation was continued up to 60 batches and 90 % of xylose utilization was observed with the formation of 4.1 g l^{-1} biomass which was 4.87-fold higher than the native strain (Fig. 1). Moreover, DCW of the strain was 2.5-fold higher as compared to the native strain. However, there was no significant improvement in sugar utilization after 50 cycles. Sonderegger and Sauer [43] generated two evolutionary strains of recombinant *S. cerevisiae* through adaptation. In another study, Liu and Hu [31] constructed a *S. cerevisiae* strain through combined approach of genetic

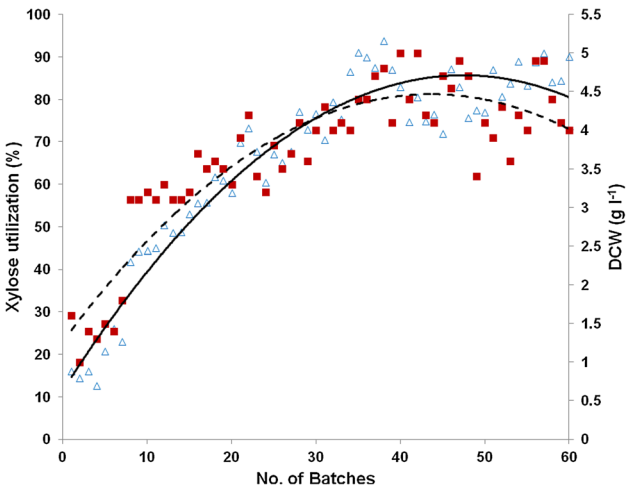

Fig. 1 Adaptation of *K. marxianus* NIRE-K1 in YEP medium containing 20 g l^{-1} xylose for 60 batches; (*empty triangle*) xylose utilization; (*filled square*) dry cell weight; (*line*) trend line of xylose utilization; (*dashed line*) trend line of DCW

engineering, mutation and adaptation for efficient xylose utilization. The developed strain showed the significant improvement in xylose utilization capacity with higher specific growth rate (0.225 h^{-1}) as compared to the native recombinant strain (0.055 h^{-1}) under aerobic condition with xylose as the carbon source. Thus, successful adaptation of yeasts *K. marxianus* NIRE-K1 to xylose containing medium suggests that this is a feasible approach to increase its performance.

Comparative growth analysis of native and adapted *K. marxianus* NIRE-K1

For a comparative analysis, native and adapted *K. marxianus* NIRE-K1 were further cultivated for 24 h in aerobic shake-flasks with YEPX medium. The performance of growth pattern in each cycle of the native and adapted yeast strain is shown in Fig. 2. There was significant difference in maximum specific growth rate between the adapted (0.097 h^{-1}) and native (0.043 h^{-1}) strain, which was 2.25-fold higher than that of native strain. However, duration of lag phase was found to be shorter in case of adapted strain (8 h) as compared to the native strain (11 h) (Fig. 2). The length of the lag time of an organism within an environment depends on several factors, including the temperature at which the microbial cells were previously incubated, source, stress conditions and the physiological state of the organism [44–46]. Mihoub et al. [47] recommended that adapted microorganisms to the new condition have much shorter lag phase than organisms that have not been previously adapted and grown at optimal conditions. In another study, Landaeta et al. [48] adapted flocculent *S. cerevisiae* (NRRL Y-265) to several inhibitory compounds found in lignocellulosic hydrolysates (acetic acid, furfural, hydroxymethylfurfural, vanillin, syringaldehyde, and hydroxybenzoic acid) to minimize the effect of inhibitors on yeast cells. They found a reduced lag phase and a higher biotransformation rate as compared to the native strain in inhibitor-containing media. Also, Koppram et al. [33] followed evolutionary engineering strategies to improve the inhibitor tolerance in the metabolically engineered xylose utilizing *S. cerevisiae* strain, TMB3400 through a repeated batch culture and resulted increase in maximum specific growth rate from 0.18 to 0.33 h^{-1} and shorten lag phase from 48 h to 24 h. Further, xylose transport rate was 3-fold higher for adapted strain (0.38 ± 0.02 g g^{-1} h^{-1}) as compared to the native strain (0.14 ± 0.04 g g^{-1} h^{-1}) (Table 1). Slininger et al. [49] adapted native pentose fermenting yeast *S. stipitis* strain NRRL Y-7124 which performed very well in hydrolyzates of high solids loading ammonia fiber expansion-pretreated corn stover (18 % w/v solids) and dilute sulfuric acid-pretreated switchgrass (20 % w/v solids). Several improved features like reduced initial lag phase, enhanced fermentation rates, improved ethanol tolerance and yield was observed through adaptation by the researchers. In the present study, evolutionary adaption also resulted in an improved aerobic growth in adapted strain on xylose as the sole carbon source. Furthermore, the effect of evolutionary adaptation on glucose utilization was examined for both native and adapted strains (Fig. 3). However, there was no significant change was observed on glucose utilization by adapted strain. The maximum specific growth rate (μ_{max}) for native and adapted *K. marxianus* NIRE-K1 were almost same as 0.24 ± 0.02, 0.23 ± 0.04 h^{-1}, respectively. On the other hand, the adapted strain showed higher ethanol yield ($Y_{P/S}$) 0.40 ± 0.001 g g^{-1}, than that of native *K. marxianus* NIRE-K1 i.e. 0.35 ± 0.02 g g^{-1} in using YPD medium (Table 1).

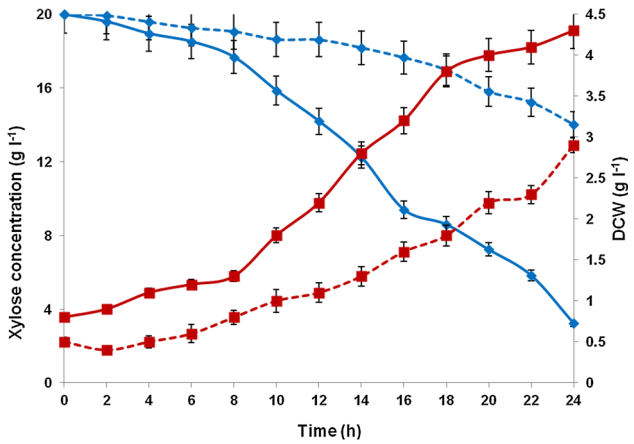

Fig. 2 Comparison of growth pattern between native (*dashed line*) and adapted (60 batches) *K. marxianus* NIRE-K1 (*line*) using xylose as a carbon source; (*filled diamond*) residual xylose; (*filled square*) dry cell weight

Fermentation of xylose using native and adapted *K. marxianus* NIRE-K1

Fermentation profile of native and adapted *K. marxianus* NIRE-K1 was evaluated under anaerobic conditions in YEP medium with initial xylose concentration of 30 g l^{-1}. The batch fermentation profile for the production of xylitol and ethanol with xylose utilization using the native and adapted strain of *K. marxianus* NIRE-K1 is shown in Fig. 4. In the present study, xylitol production by both the native and adapted cells was observed. During initial phase 0.619 ± 0.14 and 0.074 ± 0.01 g l^{-1} of xylitol was produced, using 2.42 ± 0.38 and 1.179 ± 0.33 g l^{-1} of xylose in 18 h of fermentation using adapted and native strain, respectively. Similarly, in the same duration of fermentation, the ethanol production was observed as 0.025 ± 0.01 and 0.008 ± 0.001 g l^{-1} ethanol using

Table 1 Growth kinetics of native and adapted *K. marxianus* NIRE-K1

Kinetic parameters	*K. marxianus* NIRE-K1		*K. marxianus* NIRE-K1 Ad	
Growth (glucose)	Glucose	Xylose	Glucose	Xylose
Maximum specific growth rate (μ_{max}, h^{-1})	0.23 ± 0.04	0.043 ± 0.005	0.24 ± 0.002	0.097 ± 0.01
Ethanol yield ($Y_{P/S}$, g g^{-1})	0.35 ± 0.02	0.01 ± 0.001	0.40 ± 0.001	0.02 ± 0.003
Xylitol yield ($Y_{P/S}$, g g^{-1})	–	0.076 ± 0.006	–	0.25 ± 0.02
Cell yield ($Y_{X/S}$, g g^{-1})	0.29 ± 0.01	0.40 ± 0.01	0.29 ± 0.01	0.21 ± 0.009

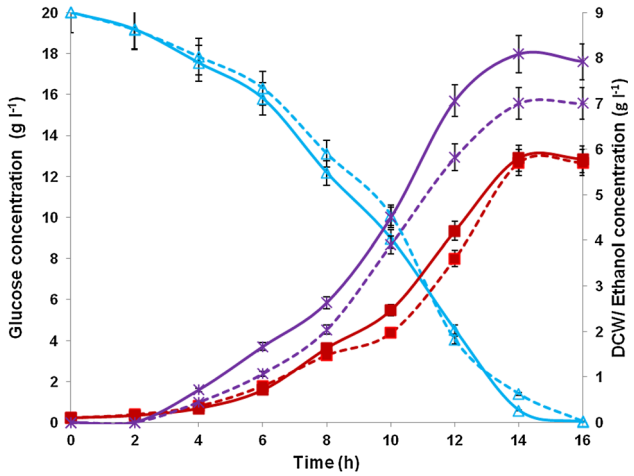

Fig. 3 Comparison of growth pattern between native (*dashed line*) and adapted (60 batches) *K. marxianus* NIRE-K1 (*line*) using xylose as a carbon source; (*empty triangle*) residual glucose; (*filled square*) dry cell weight

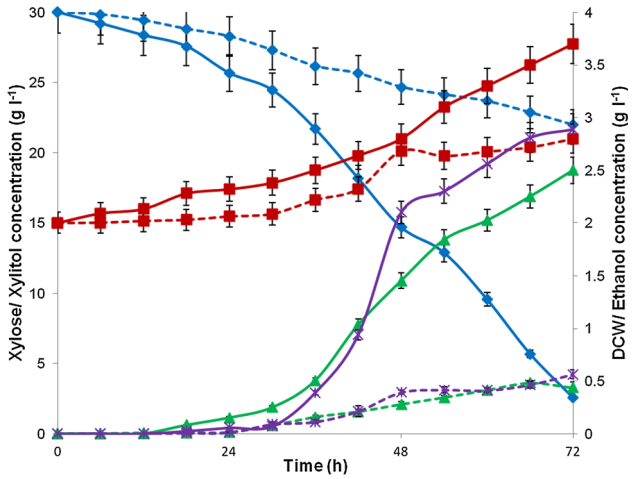

Fig. 4 Fermentation profile using optimized medium concentration in bench-scale bioreactor, (*dashed line*) native *K. marxianus* NIRE-K1; (*line*) adapted *K. marxianus* NIRE-K1; (*filled diamond*) xylose; (*filled square*) dry cell weight; (*filled circle*) ethanol; (*filled triangle*) xylitol

adapted and native strain, respectively (Fig. 4). The decrease in sugar concentration might be due to its utilization for initial growth and metabolism of the yeast in addition to its conversion into xylitol and ethanol [8, 41]. After 42 and 48 h of fermentation, 11.83 ± 1.91 and 15.31 ± 0.86 g l^{-1} of xylose was utilized with the simultaneous increase in xylitol concentration to 7.76 ± 0.97 and 10.90 ± 2.1 g l^{-1}, respectively with the cells of adapted *K. marxianus* NIRE-K1. During the same duration of fermentation using native yeast *K. marxianus* NIRE-K1, 4.34 ± 0.45 and 5.32 ± 0.37 g l^{-1} of xylose was utilized with the production of 1.57 ± 0.43 and 2.10 ± 0.43 g l^{-1} of xylitol, respectively (Fig. 4). Finally, maximum xylitol production of 18.75 ± 1.10 and 3.28 ± 0.60 g l^{-1} was achieved with utilization of 27.43 ± 2.20 and 8.02 ± 0.54 g l^{-1} of xylose in 72 h of fermentation using the cells of adapted and native *K. marxianus* NIRE-K1 (Fig. 4). Similarly, 2.88 ± 0.21 g l^{-1} of ethanol concentration was produced using the adapted yeast which was 5.11-fold higher than the native cells (0.57 ± 0.09). This study concluded that the sugar utilization and product formation were higher in adapted cells of *K. marxianus* NIRE-K1.

Fermentation kinetics of native and adapted strains of *K. marxianus* NIRE-K1 has reported in Table 2. The xylitol concentration (P) of adapted *K. marxianus* NIRE-K1 (18.75 ± 1.10 g l^{-1}) was 5.71-fold higher than that of native strain (3.28 ± 0.60 g l^{-1}), whereas the volumetric substrate uptake (Q_S) was found to be 3.45-fold higher in case of adapted *K. marxianus* NIRE-K1 (0.38 ± 0.03 g l^{-1} h^{-1}) than that of native strain (0.11 ± 0.02 g l^{-1} h^{-1}). The xylitol yield ($Y_{P/S}$ = 0.68 ± 0.05 g g^{-1}) and volumetric product productivity (Q_P = 0.260 ± 0.04 g $l^{-1}h^{-1}$) using the adapted cells of *K. marxianus* NIRE-K1 were found to be 1.65 and 5.78-fold higher than that of native *K. marxianus* NIRE-K1 cells as $Y_{P/S}$ (0.41 ± 0.05 g g^{-1}) and Q_P (0.045 ± 0.02 g g^{-1}). The ethanol yield ($Y_{P/S}$ = 0.11 ± 0.02 g g^{-1}) using adapted *K. marxianus* NIRE-K1 was found to be 1.57-folds higher than that of $Y_{P/S}$

Table 2 Fermentation kinetics of native and adapted *K. marxianus* NIRE-K1

Kinetic parameters	*K. marxianus* NIRE-K1	*K. marxianus* NIRE-K1 Ad
Ethanol ($P_{ethanol}$, g l^{-1})	0.57 ± 0.09	2.88 ± 0.21
Xylitol ($P_{xylitol}$, g l^{-1})	3.28 ± 0.38	18.75 ± 0.96
Cell yield ($Y_{X/S}$, g g^{-1})	0.099 ± 0.02	0.062 ± 0.004
Ethanol yield ($Y_{P/S}$, g g^{-1})	0.07 ± 0.01	0.11 ± 0.02
Xylitol yield ($Y_{P/S}$, g g^{-1})	0.41 ± 0.05	0.68 ± 0.05
Volumetric substrate uptake (Q_S, g l^{-1} h^{-1})	0.11 ± 0.02	0.38 ± 0.03
Volumetric product productivity (Ethanol) (Q_P, g l^{-1} h^{-1})	0.008 ± 0.002	0.040 ± 0.003
Volumetric product productivity (Xylitol) (Q_P, g l^{-1} h^{-1})	0.045 ± 0.02	0.260 ± 0.04
Specific sugar consumption rate (q_S, g g^{-1} h^{-1})	0.013 ± 0.005	0.063 ± 0.01
Specific product formation rate (Ethanol) (q_P, g g^{-1} h^{-1})	0.002 ± 0.001	0.014 ± 0.002
Specific product formation rate (Xylitol) (q_P, g g^{-1} h^{-1})	0.01 ± 0.004	0.09 ± 0.02
Conversion rate (%) into ethanol	14.09 ± 2.67	21.06 ± 2.31
Conversion rate (%) into xylitol	40.91 ± 4.61	68.36 ± 7.66

(0.07 ± 0.01 g g^{-1}) of native yeast cells. Likewise, the final sugar to ethanol and xylitol conversion rates (%) with adapted *K. marxianus* NIRE-K1 cells were 33.10 and 40.16 %, which were more than that of native strain. Similar results were found by several researchers in support to their fermentation kinetics followed by effective adaptation process. Nigam [50] studied fermentation kinetics through the adaptation of *S. stipitis* in acid hydrolysate and developed a mutant strain having ability to ferment acid hydrolysate in shorter fermentation time and better tolerance to acid. After adaptation, the ethanol yield ($Y_{P/S}$) and productivity (Q_P) of strain were increased 1.6 and 2.1-folds, respectively. Similarly, Martin et al. [51] adapted xylose-utilizing genetically engineered strain of *S. cerevisiae* for 353 h to inhibitors (phenolic compounds, furaldehydes and aliphatic acids) in bagasse hydrolysate and reported the increased performance of ethanol yield as 0.38 g g^{-1} for adapted strain as compared to 0.18 g g^{-1} for native strain. The specific ethanol productivity increased to 2.55 g g^{-1} h^{-1} by the adapted strain as compared to 1.15 g g^{-1} h^{-1} in by native strain. Furthermore, Pereira et al. [52] followed evolutionary engineering approach to obtain a variants strain of *S. stipitis* with increased tolerance to hardwood spent sulfite liquor (HSSL) containing high amount of xylose and inhibitors. The ability of the adapted strain increased from 20 to 60 % (v/v) HSSL concentration with higher substrate uptake rate (0.22 g l^{-1} h^{-1}), 7 % higher ethanol efficiency and improved ethanol yield (0.16 g g^{-1}). From above observations, it was found that the adapted culture was more suitable, since it could ferment xylose in a shorter period and gave a better yield than its native strain. This proves the success of adaptation process in the near future for the bioethanol production from lignocellulosic materials through the improvement of co-fermentation of different source of sugars by enhancing tolerance to high temperature and inhibitors.

Several researchers adopted the process of adaptation to enhance the growth and fermentation rates. Silva et al. [53] increased 3-fold of xylitol yield by using the cells of *Candida guilliermondii*, which was previously adapted in rice straw hydrolysate. Adaptation strategy was also used to stabilize the mutation in recombinant strains. Recombinant *S. cerevisiae*, with Xyl1 and Xyl2 genes of *S. stipitis* was adapted in xylose as sole carbon source which resulted in 4.75-fold higher xylose utilization with 11 % increase in ethanol production [31]. Evolutionary adaptation of recombinant shochu yeast increased anaerobic growth on xylose containing medium of adapted strain M1 with 2-fold increase in xylose consumption and ethanol production [54]. A long term evolutionary adaptation was applied on *S. cerevisiae* for increased gluconate assimilation. Evaluation by ^{13}C metabolic flux analysis revealed 6 % higher relative flux in pentose phosphate pathway for adapted strain [32]. Diao et al. [40] applied adaptive evolution on genetically modified strain with Gxf1 from *C. intermedia*, XylA from *piromyces sp*. and overexpression of endogenus genes including Xks1, Rki1, Rpe1, Tkl1, and Tal1. The developed strain could utilize 80 g l^{-1} of glucose and 40 g l^{-1} of xylose in 24 h with the production of 53 g l^{-1} ethanol. *S. cerevisiae* engineered with XylA, Xks1, Gre3, Pho13 and Tal1, was adapted in xylose rich medium. The evolved strain (SXA-R2P-E) efficiently utilized xylose and produced ethanol with an ethanol yield 0.45 g g^{-1} [35]. Further, Agbogbo et al. [37] concluded that adaptation of *S. stipitis* CBS 6054 in solid agar and reported to produce

19.4 g l^{-1} ethanol concentration as compared to 18.4 g l^{-1} in liquid hydrolyzates adapted and 16.3 g l^{-1} with native yeast [37]. Wahlbom et al. [55] compared *S. stipitis* CBS 6054 with novel recombinant *S. cerevisiae* TMB 3399 and TMB 3400 for xylose utilization through cultivation under aerobic, oxygen-limited, and anaerobic conditions, where *S. stipitis* CBS 6054 showed 0.44 ± 0.02 h^{-1} and 0.57 ± 0.025 g g^{-1} maximum specific growth rate (μ_{max}), and biomass yield ($Y_{X/S}$), respectively under aerobic condition. However, it has been reported to be high ethanol formation with less xylitol under anaerobic condition i.e. ethanol and xylitol yields 0.40 ± 0.064 and <0.01 g g^{-1}, respectively [55]. In the present study, the adapted *K. marxianus* NIRE-K1 showed maximum specific growth rate (μ_{max}) and biomass yield ($Y_{X/S}$) as 0.097 ± 0.01 h^{-1} and 0.21 ± 0.009 g g^{-1}, respectively under aerobic condition (Table 1), whereas, the ethanol and xylitol yields were calculated as 0.11 ± 0.02 and 0.68 ± 0.05 g g^{-1}, respectively under anaerobic condition (Table 2).

Conclusions

Fermentation with the adapted strain of *K. marxianus* NIRE-K1 revealed a considerable decrease in lag phase and increase in the ethanol and xylitol productivity, which is beneficial from an economic point of view. The experimental analyses suggested that the adapted strain was able to convert xylose to ethanol at a faster rate than the native strain. It is possible to use higher concentration of xylose in the medium for further improvement of the yeast strain in the subsequent batches of adaptation. However, further studies are required for the fermentation of lignocellulosic hydrolysate using the adapted strain of *K. marxianus* NIRE-K1 and to decreases the need for more extensive measures against problems with fermentation inhibitors.

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