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Performances of *Lactobacillus brevis* for Producing Lactic Acid from Hydrolysate of Lignocellulosics

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Abstract Utilizing all forms of sugars derived from lignocellulosic biomass via various pretreatment and hydrolysis process is a primary criterion for selecting a microorganism to produce biofuels and biochemicals. A broad carbon spectra and potential inhibitors such as furan, phenol compounds and weak acids are two major obstacles that limited the application of dilute-acid hydrolysate of lignocellulosics in lactic acid fermentation. Two strains of bacteria isolated from sour cabbage, S3F4 (*Lactobacillus brevis*) and XS1T3-4 (*Lactobacillus plantrum*), exhibited the ability to utilize various sugars present in dilute-acid hydrolysate of biomass. The S3F4 strain also showed strong resistance to potential fermentation inhibitors such as ferulic acid and furfural. Fermentation in flasks by this strain resulted in 39.1 g/l of lactic acid from dilute acid hydrolysates of corncobs that had initial total sugar concentration of 56.9 g/l (xylose, 46.4 g/l; glucose, 4.0 g/l; arabinose, 6.5 g/l). The hydrolysate of corncobs was readily utilized by S3F4 without detoxification, and the lactic acid concentration obtained in this study was higher compared to other reports.

Keywords Lactic acid · Lignocellulose · Dilute acid hydrolysate · Inhibition · Fermentation

Introduction

Lactic acid has a wide range of applications in food, pharmaceutical, and cosmetic industries. Lactic acid is also an important platform chemical [1, 2] as it can be converted into various chemicals such as acrylic acid, propylene glycol, acetaldehyde, and 2,3-pentanedione. In recent years, demands for lactic acid have increased due to its application

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in biodegradable polymers and green solvents [3, 4]. Lactic acid can be produced either by fossil oil-derived chemical synthesis or by fermentation. The environmental concern associated with chemical synthesis and the finite nature of petroleum supply have accelerated the development of fermentation production platform. Currently, lactic acid is commercially produced from fermenting glucose, starch, liquefied starch, or sucrose. It is estimated in lactic acid fermentation that 60~80% [5–7] of the production cost is related to raw materials. Using low cost raw materials such as lignocellulosic biomass, the largest known renewable carbohydrate resources is required for building a substantial renewable lactic acid industry. Accordingly, considerable efforts have been directed to search for organisms capable of fermenting lignocellulosic hydrolysates into lactic acid.

Breaking the biopolymers into simple sugars through pretreatment and hydrolysis is a prerequisite for lignocellulosics utilization. Lignocellulosics is typically composed of 38~50% cellulose, 17~32% hemicellulose, and 15~30% lignin [8]. Hemicellulose, which mainly consists of pentose sugars, is more easily degradable compared to cellulose and lignin. Among the pretreatment and hydrolysis processes for lignocellulosics, dilute-acid pretreatment was generally regarded as one of the most economic and efficient method [9]. Dilute-acid pretreatment efficiently hydrolyzes hemicellulose to xylose, arabinose and glucose, and the process enables further enzymatic digestion of cellulose to glucose.

Another obstacle for the use of lignocellulosic hydrolysates is the inhibition effect of toxic compounds released during the pretreatment process. In addition to the fermentable sugars, compounds that are toxic to fermentative organisms such as furfural, phenolic derivatives, and inorganic acids were also produced in this process. For example, more than 35 potentially inhibitory compounds have been identified in acid hydrolysates [10, 11]. The severe inhibitory effect of furans, phenols, and organic acids produced during dilute-acid hydrolysates has been widely studied in ethanol fermenting microorganisms. In general, they affect overall cell physiology and often resulted in decreased viability, ethanol yield, and productivity. Although detoxification methods such as bioabatment [12, 13] and over-limming [14] have been proposed, developing strains with resistance to inhibitors is the best approach to resolve the problem from an economic standpoint.

Due to the complex compositions of biomass and associated by-products from the pretreatment and hydrolysis of the biomass, discovery of strains with abilities to not only ferment various sugars released, but also resist fermentation inhibitors is of great interest to the researchers. Since industrial lactic acid producing bacteria generally lack the ability to ferment xylose, which is an important component of lignocellulosic hydrolysis. Efforts have been reported on the utilization of lignocellulosic hydrolysates by some *Lactobacillus* [15–18], *Bacillus* [19–22], and metabolically engineered *E. coli* [17]. Additionally, solutions to problems such as catabolic repression [15, 17] and sensitivity to toxic compounds [15, 17, 19, 21] were explored in the fermentation of lignocellulosic hydrolysates. However, an ideal organism has not been found.

As an attempt to develop better performers of lactic acid fermentation from biomass-derived sugars, we isolated two *Lactobacillus* strains, S3F4 and XS1T3-4, from sour cabbage in non-detoxified dilute acid hydrolysates of corn stover as the selection medium. We first identified these two strains as *Lactobacillus brevis* (S3F4) and *Lactobacillus plantrum* (XS1T3-4), based on the comparison of 16S rRNA sequences. Then, we conducted experiments testing the ability of these two strains in using various monosaccharides presumably present in lignocellulose materials. We further tested the tolerance capability of these strains to the potential inhibitors presented in the dilute-acid hydrolysate of corn stover and corncobs.

Materials and Methods

Microorganism and Media

L. brevis (S3F4) and *L. plantrum* (XS1T3-4) isolated according to the below procedure were employed in this work. The selection medium used for the initial isolation of bacteria resistant to toxic compounds in acid hydrolyzed lignocellulosic material contained the following per liter of dilute-acid hydrolysate of corn stover: 10 g acid hydrolyzed casein, 5 g yeast extract, 1 g Tween 80, 2 g diammonium citrate, 2 g K_2HPO_4, 2 g $NaCH_3CO_2$, 0.2 g $MnSO_4 \cdot H_2O$, 0.05 g $MgSO_4 \cdot 7H_2O$. Deman Rogosa-Sharp (MRS) medium with 20 g/l of xylose (MRSX) as carbon source was used to select for xylose fermentation strains. Solid medium contained 1.8% agarose.

Medium used for reselection strains that could produce lactic acid from xylose (MRSXB) is MRS with 20 g/l xylose as carbon source and 1% bromocresol green. The fermentation medium contained 10.0 g/l peptone, 10.0 g/l yeast extract, 6.0 g/l beef extract, 0.2 g/l $MgSO_4$, 0.2 g/l $MnSO_4$, 0.03 g/l NaCl, 0.01 g/l $FeSO_4$, 4.0 g/l $NaCH_3CO_2$, 2.0 g/l diammonium citrate, 2.0 g/l KH_2PO_4, 1.0 ml/l Tween 80, and the carbohydrate was added separately according to the experiment. For lignocellulosic fermentation, a ×10 concentrated nutrient medium (MRS without carbon source) was added to the hydrolysate. All experiments were performed in duplicate.

Isolation of Strains

Environmental samples collected from different resources were first suspended in 50 ml of selection medium for 12 h (160 rpm and 30 °C). The enriched samples were plated on the MRSXB medium. With the added acid indicator bromocresol green, the color of the medium around the colonies will be changed from green into yellow by acid producing strains. After 48 h of incubation, representative colonies with yellow color-changing circle were selected and stored for further analysis.

The selected colonies were incubated in the selection medium and MRSX medium for 24 h, and the fermentation broth was centrifuged and filtered to dispose of the cell mass. Thin layer chromatography was then used to determine lactic acid concentration in the fermentation broth.

After several rounds of selection, two lactic acid producing strains (S3F4 and XS1T3-4) were isolated from sour cabbage samples that grew well in the corn stover hydrolysate and selected for further analysis. 16S rRNA sequence analysis indicated that S3F4 showed 99% 16S rRNA gene sequence similarity to that of *L. brevis*, and XS1T3-4 showed 99% 16S rRNA gene sequence similarity to that of *L. plantrum*.

Preparation of Dilute-Acid Hydrolysate of Lignocellulosics

Dilute-Acid Hydrolysate of Corn Stover [23]

Mashed corn stover was suspended in 2% sulfuric acid at a 10% (*w*/*v*) loading, the mixture was heated to 121 °C in the autoclave, and incubated for 1 h. Solids were removed by centrifugation for 10 min at 15,000×*g*. The pH of the supernatant was adjusted to 7.0 with calcium hydroxide, the precipitate was removed by filtration, the collected supernatant was autoclaved at 110 °C for 20 min, and the solution was stored at room temperature for use.

Dilute-Acid Hydrolysate of Corncobs [24]

A 100 g mashed corncobs were first suspended in 600 ml of 0.1% sulfuric acid, maintained at 80°C with whisking for 1 h, the sulfuric acid concentration was then adjusted to 0.8%, and the mixture was heated to 110°C for 2 h. After that, the same procedure as used for corn stover was followed.

According to the NREL methods [25], if the biomass is completely hydrolyzed, the concentration of cellobiose should be less than 3 mg/ml. In this work, cellobiose in the two kinds of hydrolysates were not detected by HPLC analysis, which means the absence of oligomeric sugars.

Batch Fermentation

Although glucose, xylose, and arabinose are the main components of lignocellulosic materials, other monosaccharides and disaccharides such as mannose, galactose, and cellobiose are also produced in the lignocellulosic hydrolysates, and the concentration are varied with the biomass resources and pretreatment methods [26, 27]. It is therefore necessary to clarify the carbohydrate utilization pattern of the isolated strains. Considering the lignocellulosic composition, S3F4 and XS1T3-4 were tested for growth and ability to produce lactic acid from different lignocellulosic carbohydrates.

For carbohydrate pattern test, the microorganisms were first spread on plates containing MRS medium, and then maintained at 30°C for 48 h. Well-formed single colonies were inoculated on MRS plates with different carbon sources(20 g/l of glucose, xylose, arabinose, mannose, galactose, cellobiose, xylitol, arabitol, and lactose separately) and 1% bromocresol green as acid indicator.

Inhibition phenomena have also been reported for *Lactobacillus casei* subspecies *rhamnous* [15], *Bacillus* subspecies [19, 21], *Lactobacillus pentosus* [18], and *Escherichia coli* [17] in lactic acid fermentation, presumably from dilute acid hydrolysates of lignocellulose. To verify the resistance of the isolated strain S3F4 to inhibitors from the corn stover dilute-acid hydrolysate without detoxification, typical inhibitors such as furfural and ferulic acid were added to the culture medium, and the inhibitory effect on cell viability and lactic acid yield and productivity of S3F4 from xylose was investigated.

For growth test of S3F4 with glucose and xylose, and typical inhibitors (furfural and ferulic acid), 200 µl culture medium in a 96-well plates was inoculated with 1% seed broth. Samples were maintained in micro-plate spectrophotometer (Spectramax 190, Molecular Devices Corp., Sunnyvale, CA) at 30°C for 20 h, and the OD_{600} was recorded every 30 min. Batch fermentation was conducted at 30°C in a 100-ml flask with a 50 ml of working volume at 160 rpm. Fermentation was initiated by adding a 5% (*v*/*v*) inoculum.

Analysis

Glucose, xylose, arabinose, lactic acid, and acetic acid were measured via HPLC using a 2414 refractive index detector (Waters, Milford, MA, USA) and an IC-Pak Ion Exclusion column 7.8 mm×300 mm (Waters, Milford, MA, USA). Samples were eluted using 2 mM H_2SO_4 at a flow rate of 0.6 ml/min at 50°C.

Results and Discussion

Fermentation Performance on Different Carbohydrates

The experimental results on utilization of different sugars are shown in Table 1. S3F4 was able to grow and produce acid from glucose, xylose, and arabinose which are the predominant components in lignocellulosic hydrolysates. XS1T3-4 could utilize glucose and arabinose to produce acid, but the growth on xylose was much weaker and no acid production was observed. The sugars resulted from the hydrolysate of the lignocellulosics varied with the category of raw materials and hydrolysis methods [26, 27], and no single strain was found to be able to fully utilize all the sugars in the hydrolysates. While, co-cultivation of different strains with different carbohydrate spectrum is possible to fully utilize all the sugars in the hydrolysates. The carbon spectrum test showed that S3F4 and XS1T3-4 possessed a compensated carbohydrate spectrum, which indicates the potential of co-cultivation of the two strains in different lignocellulosic hydrolysates. This is consistent with the observation by other researchers as it was reported that lactic acid production increased to 95% of the theoretical yield by co-cultivation of *L. brevis* and *L. pentosus* with compensated carbohydrates spectrum, and approximately 34% and 7% higher yields than from separate fermentation of the two stains from wheat straw hemicellulose hydrolysates [16].

Figure 1 depicts the growth performance of S3F4 and XS1T3-4 in the 96-well plates with xylose, glucose, corn stover, and corncobs hydrolysates as carbon source without pH control. S3F4 had a better performance on xylose than glucose. Although the specific growth rate of S3F4 on the hydrolysate was lower than xylose, the final cell concentration (OD_{600}) was similar at approximately 1.2. XS1T3-4, in contrast to S3F4, showed great performance on glucose. The cell density (OD_{600}) reached 1.6 with glucose and glucose plus xylose as carbon sources, while with xylose as the carbon source, the cell density (OD_{600}) only reached 0.4. Although the xylose fermentation ability of XS1T3-4 was poor, solid medium tests indicated that it grew well with cellobiose and mannose, which were poorly utilized by S3F4.

Table 1 Carbon spectrum information for S3F4 and XS1T3-4 for lignocellulosic hydrolysates.

Carbon source	S3F4	XS1T3-4
Glucose	+++, A	+++, A
Xylose	+++, A	++
Arabinose	+++, A	+++, A
Mannose	++	+++, A
Galactose	+++, A	+++, A
Cellobiose	++	+++, A
Xylitol	+	++
Arabitol	+	++
Lactose	++	+++, A

Results shown were the average of duplicate runs

+++ Strong growth, *A* Acid producing, ++ moderate growth, + weak growth

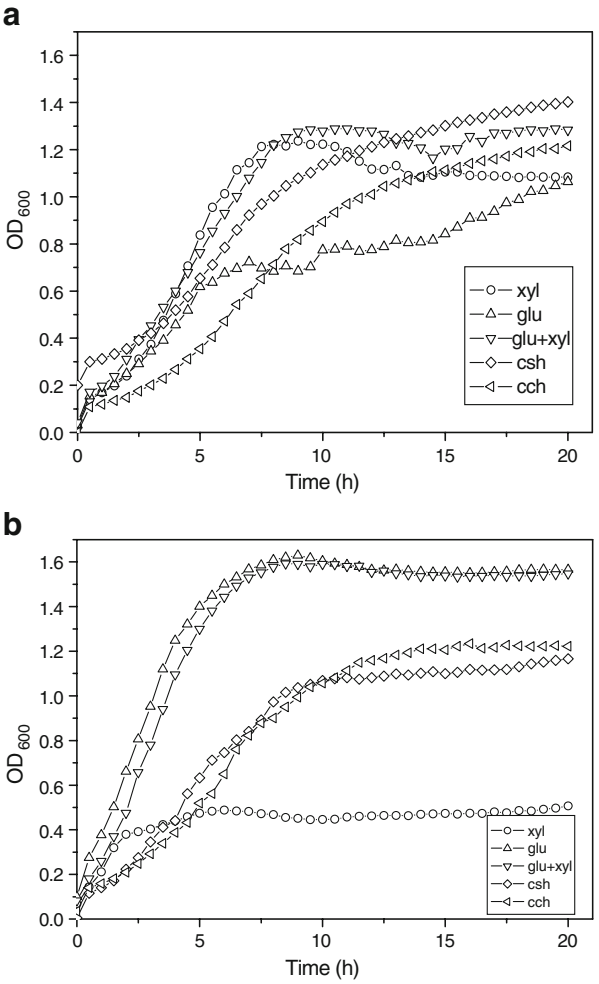

Fig. 1 Growth curve of S3F4 and XS1T3-4 with different carbon source. Results shown were the average of duplicate runs. **a** S3F4; **b** XS1T3-4. *xyl* 25 g/l xylose, *glu* 25 g/l glucose, *glu+xyl* 12.5 g/l glucose and 12.5 g/l xylose, *csh* corn stover dilute-acid hydrolysate, *cch* corncobs dilute-acid hydrolysate

Tolerance of S3F4 to the Inhibitors in Dilute-Acid Hydrolysates

The growth curve and lactic acid fermentation results of S3F4 with the medium with inhibitors are shown in Fig. 2 and Table 2. Compared with the control, the growth rate of S3F4 with 5 and 10 mM ferulic acid was similar and the cell density (OD_{600}) at the stationary phase was 0.2 higher, which means ferulic acid at these two concentrations even promoted the growth of S3F4. 15 mM ferulic acid prolonged the onset of the stationary phase from 7 to 12 h. Furfural at 10, 15, and 20 mM, on the other hand, had slight inhibitory effects on the growth of S3F4. Ferulic acid and furfural together had a notably worse effect on the growth of S3F4 from xylose. The onset of the stationary phase of S3F4

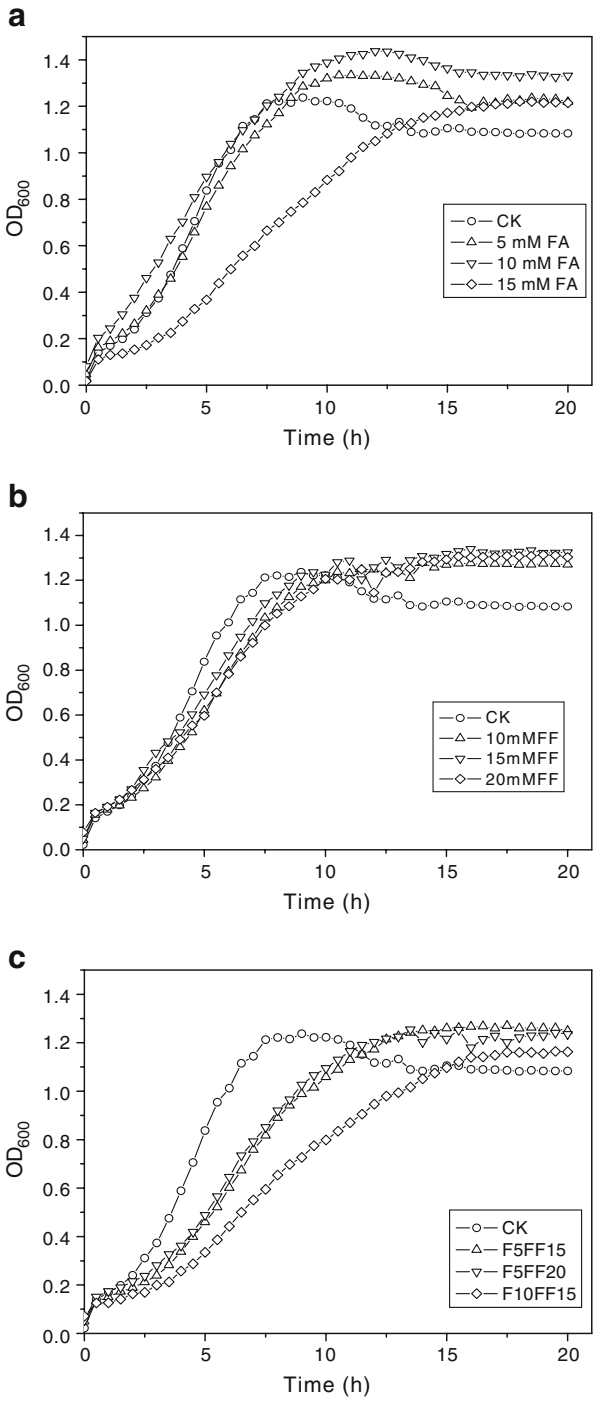

Fig. 2 Growth curve of S3F4 with xylose as carbon source (25 g/l) in the presence of inhibitors. Results shown were the average of duplicate runs. **a** ferulic acid; **b** furfural; **c** ferulic acid and furfural; *CK* control, *F5FF15* 5 mM ferulic acid and 15 mM furfural, *F5FF20* 5 mM ferulic acid and 20 mM furfural, *F10FF15* 10 mM ferulic acid and 15 mM furfural

Table 2 Lactic acid fermentation from xylose of S3F4 with the addition of inhibitors.

Inhibitors		Time (h)	Xyl (g/l)[a]	Lac_{max} (g/l)	Ace (g/l)
Control		30 h	0.2±0.1	17.4±0.6	10.0±0.1
Ferulic acid	5 mM	24 h	0.2±0	17.6±0.4	10.3±0.3
	10 mM	24 h	0.2±0	18.2±0.8	10.4±0.5
	15 mM	24 h	12.4±2.0	9.2±1.4	5.5±1.5
Furfural	10 mM	24 h	0.1±0	18.4±1.0	10.7±1.0
	15 mM	24 h	0.2±0	18.6±0.7	10.3±0.1
	20 mM	24 h	0.2±0	18.9±0.1	10.5±0.3
Ferulic acid+furfural	5 mM+15 mM	31 h	0.3±0.1	17.6±0.7	9.2±0.2
	5 mM+20 mM	31 h	0.2±0.1	16.9±0.8	8.2±0.8
	10 mM+15 mM	31 h	0.2±0	18.7±0.2	8.2±0.6

[a] The initial xylose concentration was 25.0 g/l

Xyl xylose, Lac_{max} max lactic acid concentration, *Ace* acetic acid concentration

was prolonged to approximately 8.5–9 h as compared with the control, when the two compounds were added to the culture medium simultaneously. The tolerance of S3F4 to the two inhibitors was better than reported pentose fermenting strains. It was reported [28] that *E. coli* was inhibited by ferulic acid at only 3 mM. Although the reported strains generally showed better resistance to furfural, *Zymomonas mobilis* and *Pichia stipititis* were inhibited by furfural at 10 mM. No inhibitory effect was observed when S3F4 was treated with 10 mM ferulic acid and 20 mM furfural separately, which indicated the strong tolerance ability of S3F4.

The xylose fermentation result shows that the highest lactic acid concentration was obtained at 24 h with ferulic acid addition at 5, 10, and 15 mM, and furfural at 10, 15, and 20 mM, which was approximately 6 h sooner than the control. Simultaneous exposure to ferulic acid and furfural has no negative or positive effect on the lactic acid fermentation ability of S3F4 from xylose, as the maximum lactic acid concentration reached at 31 h, similar to the control. This indicated that ferulic acid and furfural separately accelerated the producing rate of lactic acid from xylose to some extent. The negative effect of ferulic acid and furfural on the growth of S3F4 might be due to the low pH in the fermentation broth, as the growth curve was evaluated without pH control.

Lactic Acid Fermentation of S3F4 from Different Carbon Source

The performance of S3F4 in terms of lactic acid fermentation with respect to xylose and glucose is shown in Tables 3. The strain was able to use up all the glucose within 24 h and convert it to mainly lactic acid. When only xylose was used, it took about 30 h to consume the majority of the sugar and yield was lower. The mixed sugars of glucose and xylose resulted in the lowest lactic acid yield per unit of sugars.

L. brevis is generally regarded as an obligate heterofermentative bacterium, but the high yield of 0.86 g/g from glucose indicated that glucose was not metabolized through the Phosphoketoslase (PK) pathway, according to which only 0.5 g lactic acid should be produced from 1 g glucose. This result is in accordance with that of Garde [16], who

Table 3 Lactic acid production of S3F4 from glucose and xylose.

Substrate	Time (h)	Glu (g/l)	Xyl (g/l)	Lac (g/l)	Ace (g/l)	Q_{Lac} ($gl^{-1}h^{-1}$)	Q_{Ace} ($gl^{-1}h^{-1}$)	$Y_{Lac/s}$ (g/g)	$Y_{Ace/s}$ (g/g)
Glu[a]	24	0	–	21.6±0	3.6±0.1	0.90±0	0.15±0	0.86±0	0.14±0
Xyl[a]	30 h	–	0.4	17.4±0.6	10.0±0.1	0.58±0.02	0.33±0	0.70±0.02	0.40±0
Glu/Xyl[a]	24 h	0	0.3	16.9±0.2	7.5±0.1	0.70±0.01	0.31±0	0.68±0.01	0.30±0

Glu glucose, *Xyl* xylose, *Lac* maximum lactic acid concentration, *Ace* maximum acetic acid concentration, Q_{Lac} lactic acid volumetric productivity, Q_{Ace} acetic acid volumetric productivity, $Y_{Lac/S}$ lactic acid yield from sugars, $Y_{Ace/S}$ acetic acid yield from sugars

[a] The initial sugar concentration was 25 g/l, and the mixed sugar was 1:1 (*w*/*w*)

reported that the lactic acid yield of 89% by *L. brevis* from glucose. It was reported [29] that enzymes for the Embden-Meyerhof-Parnas (EMP) pathway are inducible in *L. brevis*, so the high yield of lactic acid from glucose reported in this paper maybe due to the EMP pathway employed by S3F4. The lactic acid yield from xylose only achieved 0.71 g/g, which was much lower than from glucose. Meanwhile it showed a high yield of acetate in comparison to glucose, indicating that xylose was mainly metabolized through PK pathway.

Carbon catabolite repression is a common phenomenon investigated in pentose fermenting microorganisms. When glucose is present in the cultivation broth, the pentose sugars will not be utilized until the glucose is depleted. The lignocellulose hydrolysates are usually composed of a mixture of glucose and pentose sugars, so the pentose sugars utilization in the hydrolysates, such as xylose and arabinose, will be delayed because of the glucose repression, and this will subsequently decrease the productivity. The catabolite repression has been reported in *E. coli* [17], *L. casei* subspecies *Rhamnous* [15] and *Rhizopus oryzae* [30]. Although catabolite repression mutants of *E. coli* had better performances in xylose and glucose mixture fermentation, the highest xylose consumption rate was only achieved 65±14% when 40 g/l of glucose and 40 g/l of xylose were used [31].

Figure 3 shows the sugar utilization pattern when glucose and xylose mixture with different concentrations were fermented by S3F4. Xylose supplied (10~40 g/l) in the fermentation broth was almost completely utilized by S3F4 as when glucose (10~40 g/l) was disappeared nearly simultaneously from the culture medium. No catabolite repression phenomenon was observed when glucose (10~40 g/l) existed in the fermentation broth. These results indicated that glucose and xylose could be simultaneously utilized by S3F4, which is expected for lignocellulosic hydrolysates fermentation, because of the complex composition of the hydrolysates.

To understand the performance of S3F4 in the lignocellulosic hydrolysates without detoxification, dilute acid hydrolysis of corn stover with 24.6 g/l total sugars and corncobs with 56.9 g/l total sugars was used for fermentation and the results were given in Table 4. The data shows that S3F4 could efficiently convert the sugars in hydrolysates into lactic acid without significant inhibition. The highest lactic acid concentration of 18.2 g/l was achieved at 24 h in corn stover and 39.1 g/l in corncobs at 48 h. At higher initial total sugar concentrations, a higher lactic acid productivity of 0.82 $gl^{-1}h^{-1}$ was achieved in corncobs hydrolysates. The higher lactic acid yield of 0.75 g/g of S3F4 in corn stover may be due to higher initial glucose concentration in the hydrolysates.

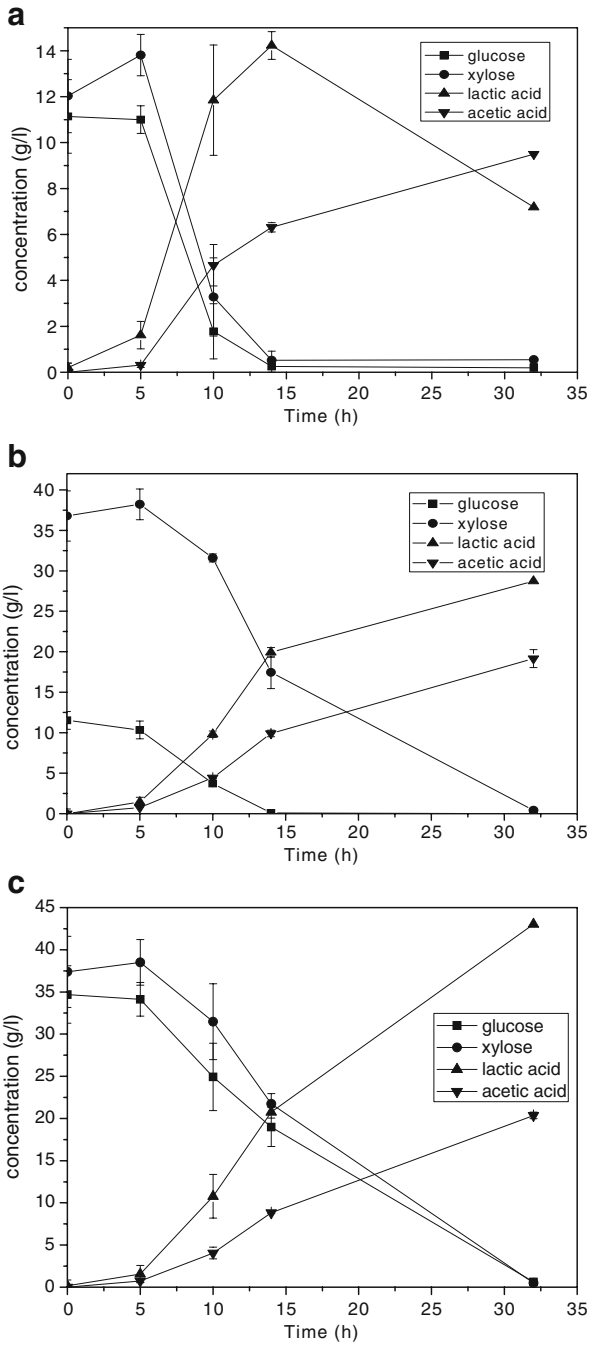

Fig. 3 S3F4 was used to ferment glucose and xylose mixture, results shown were the average of duplicate runs. **a** The carbon resource was the mixture of 12.5 g/l of glucose and 12.5 g/l of xylose. **b** The carbon resource was the mixture of 12.5 g/l of glucose and 40 g/l of xylose. **c** The carbon resource was the mixture of 40 g/l of glucose and 40 g/l of xylose

Table 4 Lactic acid production of S3F4 from dilute-acid hydrolysates of agricultural residues.

Hydrolysates		Glu (g/l)	Xyl (g/l)	Ara (g/l)	Lac (g/l)	Ace (g/l)	Q_{Lac} ($gl^{-1}h^{-1}$)	Q_{Ace} ($gl^{-1}h^{-1}$)	$Y_{Lac/s}$ (g/g)	$Y_{Ace/s}$ (g/g)
Corn stover	0 h	7.8± 0.3	14.3±0.5	2.5± 0.1	0	3.3±0.3	–	–	–	–
	11 h	2.2± 0.1	10.8±±1.5	0.25± 0	7.7±1.7	4.8±0.1	–	–	–	–
	24 h	0	0.4±0	0	18.2± 0.15	10.7±0.3	0.76± 0.01	0.31± 0.01	0.74± 0.03	0.31± 0.01
Corncobs	0 h	4.0±0	46.4±0.9	6.5± 0.1	0.1±0.1	8.7±0.1	–	–	–	–
	40 h	0	3.8±1.5	0	36.2±0	30.8±0.1	0.90±0	0.55±0	0.63± 0.01	0.39±0
	48 h	0	1.3±0.05	0	39.2±0.3	33.1±0	0.81± 0.01	0.51±0	0.69± 0.01	0.43± 0.01

Glu glucose, *Xyl* xylose, *Ara* arabinose, *Lac* maximum lactic acid concentration, *Ace* maximum acetic acid concentration, Q_{Lac} lactic acid volumetric productivity, Q_{Ace} acetic acid volumetric productivity, $Y_{Lac/S}$ lactic acid yield from sugars, $Y_{Ace/S}$ acetic acid yield from sugars

Although the concentration of the by-product acetic acid observed in the present work was high, the lactic acid concentration from lignocellulosic hydrolysates was still higher than other reported strains (Table 5).

According to Table 5, the reported highest lactic acid yield from xylose was achieved by *Bacillus coagulans* and genetically engineered *E. coli*, however, the lignocellulosic hydrolysate used in the reports had to be detoxified before fermentation, and notable inhibition phenomena were observed when total sugars in diluted acid hydrolysate reached 50 g/l. Although the lactic acid yield of *L. brevis* was lower compared with the homofermentative strains, the lactic acid yield can be enhanced by strain improvement and fermentation technology development as the reported obligate heterofermentative strain *R. oryzae* [32, 33]. Since the whole genomic sequence of *L. brevis* has been published, the isolated strain S3F4 (*L. brevis*) can in future be genetically engineered and optimized to enhance its lactic acid yield from both glucose and xylose.

Conclusion

Two isolated strains, S3F4 (*L. brevis*) and XS1T3-4 (*L. plantrum*), exhibited use of a broad carbohydrate spectrum of the monosaccharides present in lignocellulosic hydrolysates. S3F4 was especially able to simultaneously utilize xylose and glucose without catabolic repression. It also showed strong resistance to the potential inhibitors furfural and ferulic acid. It efficiently converted dilute-acid hydrolysates of corn stover and corncobs into lactic acid without detoxification. The maximum lactic acid concentration achieved was 39.1 g/l from corncobs hydrolysate with 56.9 g/l total sugars in 48 h. The lactic acid concentration was higher in comparison to other reports using lignocellulosic hydrolysates without detoxification as a carbon source.

Table 5 Comparison of lactic acid fermentation from lignocellulosic hydrolysates with other strains.

Strains	*L. brevis*	*L. brevis* and *L. pentosus*	*L. pentosus*		*L. casei* ssp. *Rhamnous*	*B. coagulancs*	*E. coli*	*Rhyzopus oryzae*
Raw materials	Corncob	Wheat straw hemicellulose	Corncobs	Barly bran husks	Soft wood	Sugar cane bagasse	Rice straw	Wheat straw
Hydrolysis process	Dilute-acid	enzymatic	Dilute-acid	Dilute-acid	Dilute-acid	Dilute-acid	Alkaline	
Total sugars (g/l)	56.9	11 ~ 12	45 ~ 50	55 ~ 60	29	81.6	58	29.5
Catabolic repression	No	n.a.	n.a.	n.a.	Yes	No	Yes	Yes
Inhibition / concentration	No	No	No	No	Yes, 40%	Yes, 50%	Yes	No
Fermentation time	48 h	24 h	60 h	60 h	132 h	192 h (60 g/l)	140 h	40 h
Lac (g/l)	39.1	8 ~ 9	24.7	33.7	22.56	55.5	49.5	6.8
Ace/Ath (g/l)	24.4	n.a.	15.3	19.0	1.24	n.a.	2	5.7
Q_{Lac} ($gl^{-1}h^{-1}$)	0.81	n.a.	0.34	0.6	n.a.	n.a.	n.a.	n.a.
Q_{Ace} ($gl^{-1}h^{-1}$)	0.51	n.a.	0.1	0.18	n.a.	n.a.	n.a.	n.a.
$Y_{Lac/s}$	0.70	0.95	0.53	0.57	0.80	0.93	n.a.	n.a.
$Y_{Ace/s}$	0.44	n.a.	0.27	0.27	n.a	n.a.	n.a.	n.a.
Reference	Present work	[16]	[18]	[18]	[15]	[21]	[17]	[30]

Lac maximum lactic acid concentration, *Ace* maximum acetic acid concentration, Q_{Lac} lactic acid volumetric productivity, Q_{Ace} acetic acid volumetric productivity, $Y_{Lac/S}$ lactic acid yield from sugars, $Y_{Ace/S}$ acetic acid yield from sugars, *n.a.* not available

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References

1. Mu, S. Y. (2001). *Chemical Techno-Economics*, 3, 10–14. (in chinese)
2. Datta, R., & Henry, M. (2006). *Journal of Chemical Technology and Biotechnology, 1*, 1119–1129.
3. Datta, R., Tsai, S. P., Bonsignore, P., Moon, S. H., & Frank, J. R. (1995). *FEMS Microbiology Reviews, 16*(2/3), 221–231.
4. Södergård, A., & Stolt, M. (2002). *Progress in Polymer Science, 27*, 1123–1163.
5. Akerberg, C., & Zacchi, G. (2000). *Bioresource Technology, 75*, 119–126.
6. Tejayadi, S., & Cheryan, M. (1995). *Applied Microbiology and Biotechnology, 43*, 242–248.
7. Oh, H., Wee, Y. J., Yun, J. S., Han, S. H., Jung, S., & Ryu, H. W. (2005). *Bioresource Technology, 96*, 1492–1498.
8. Ritter, S. K. (2008). *Plant Biochemistry, 86*(49), 15.
9. Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., et al. (2005). *Bioresource Technology, 96*, 673–686.
10. Palmqvist, E., & Bärbel, H. H. (2000). *Bioresource Technology, 74*, 25–33.
11. Mussatto, S. I., & Roberto, I. C. (2004). *Bioresource Technology, 93*, 1–10.
12. Nichols, N. N., Dien, B. S., Guisado, G. M., & Lopez, M. J. (2005). *Applied Biochemistry and Biotechnology, 121*(124), 279–390.
13. Lopez, M. J., Nichols, N. N., Dien, B. S., Moreno, J., & Bothast, R. J. (2004). *Applied Microbiology and Biotechnology, 64*, 125–131.
14. Nilvebrant, N. O., Person, P., Reimann, A., Sousa, F. D., Gorton, L., & Jonsson, L. J. (2003). *Applied Biochemistry and Biotechnology, 107*(1–3), 615–628.
15. Iyer, P. V., Thomas, S. A., & Lee, Y. Y. (2000). *Applied Biochemistry and Biotechnology, 84*(86), 665–677.
16. Garde, A., Jonsson, G., Schmidt, A. S., & Ahring, B. K. (2002). *Bioresource Technology, 81*, 217–223.
17. Shoemaker, S. (2004). Technical report, DOE contract number: FC07-99CH1007.
18. Moldes, A. B., Torrado, A., Converti, A., & Domínguez, J. M. (2006). *Applied Biochemistry and Biotechnology, 135*, 219–227.
19. Neureiter, M., Danner, H., Madzingaidzo, L., Miyafuji, H., & Thomasser, C. (2004). *Chemical and Biochemical Engineering Quarterly, 18*(1), 55–63.
20. Patel, M., Ou, M., Ingram, L. O., & Shanmugam, K. T. (2004). *Biotechnological Letters, 26*, 865–868.
21. Keelnatham T. S. (2006). Patent no. US 7098009.
22. Maas, R. H. W., & Bakker, R. R. (2008). *Applied Microbiology and Biotechnology, 78*, 751–758.
23. Zhang, Y. M., Liang, Y., Lu, X. B., Yang, J., Ma, P. S., and Zhang, S. Y. (2007) Journal of Tianjin University 40(4), 432–436. (in chinese)
24. Li, R.J., Xue, P.J., Deng, Y.D. (2007). Patent no. CN101220381.
25. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., et al. (2008). *Technical report, National Renewable Energy Laboratory*. USA: Midwest Research Institute.
26. Lee, D.K., Owens, V.N., Boe, A., Jeranyama, P. Composition of herbaceous Biomass feed stocks, Available from http://agbiopubs.sdstate.edu/articles/SGINC1-07.pdf. Accessed April 10, 2009.
27. Nilsson, A. Control of fermentation of lignocellulosic hydrolysates, Available from http://www.chemeng.lth.se/exjobb/010.pdf. Accessed March 30, 2009.
28. Klinke, H. B., Thomsen, A. B., & Ahring, B. K. (2004). *Applied Microbiology and Biotechnology, 66*, 10–26.
29. Saier, M. H., Jr., Ye, J. J., Klinke, S., & Nino, E. (1996). *Journal of Bacteriology, 178*, 314–316.
30. Mass, R. H. W., Bakker, R. R., Eggink, G., & Weusthuis, R. A. (2006). *Applied Microbiology and Biotechnology, 72*, 861–868.
31. Dien, B. S., Nichols, N. N., & Bothast, R. J. (2002). *Journal of Industrial Microbiology and Biotechnology, 29*, 221–227.
32. Bai, D. M., Zhao, X. M., Li, X. G., & Xu, S. M. (2004). *Biochemical Engineering Journal, 18*, 41–48.
33. Bai, D. M., Li, S. Z., Liu, A. L., & Cui, Z. F. (2008). *Applied Biochemistry and Biotechnology, 144*, 79–85.