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Efficient production of L-lactic acid from corncob molasses, a waste by-product in xylitol production, by a newly isolated xylose utilizing *Bacillus* sp. strain

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ABSTRACT

Lignocellulosic biomass-derived sugars are considered nowadays to be an economically attractive carbohydrate feedstock for large-scale fermentations of bulk chemicals such as lactic acid. In the present study, corncob molasses containing a high content of xylose, which is one of the lignocellulosic biomasses and a waste by-product from xylitol production, was used for L-lactic acid production via a newly isolated xylose utilizing *Bacillus* sp. strain XZL9. *Bacillus* sp. strain XZL9 can utilize the mixture of sugars including xylose, arabinose, and glucose in corncob molasses for L-lactic acid production. High concentration of L-lactic acid (74.7 g l^{-1}) was obtained from corncob molasses (initial total sugars of 91.4 g l^{-1}) in fed-batch fermentation. This study provides an encouraging means of producing L-lactic acid from lignocellulosic resource such as the low-cost corncob molasses.

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1. Introduction

Lactic acid is widely applied in the food, pharmaceutical, and cosmetic industries because of its safety properties, its optical activity, and its hydroxyl and carboxyl moieties (Yun and Ryu, 2001; Maas et al., 2008). One of its expanding uses is for synthesis of poly (lactic acid) (PLA), which is considered to be one of the most promising polymers because it can be produced from renewable resources and is biodegradable. These properties have intensified interest in developing more efficient production processes for lactic acid (Ilmen et al., 2007).

Compared to chemical synthesis, microbial fermentation is a better alternative because it leads to the production of optically pure lactic acid and offers advantages in the utilization of renewable carbohydrates (Ilmen et al., 2007). Many microorganisms have proven ability to produce lactic acid, including fungi and *Lactobacillus* species and various gene modified strains (Ishida et al., 2005; Ilmen et al., 2007). Production of lactic acid by some *Bacillus* species, including *Bacillus coagulans*, *Bacillus stearothermophilus*, and *Bacillus licheniformis*, has also been reported (Danner et al., 1998; Patel et al., 2004; Sakai and Yamanami, 2006; Maas et al., 2008; Budhavaram and Fan, 2009). As potential industrial strains, thermophilic *Bacillus* species possess remarkable advantages for lactic acid production, including simple nutrition requirements, non-sterilization fermentation, and simple maintenance of stock cultures (Michelson et al., 2006; Sakai and Yamanami, 2006; Qin et al., 2009).

To enhance the productivity and economy of lactic acid production, many extensive studies have investigated the potential of utilizing less costly raw materials, such as starchy, cellulosic materials and molasses (Dumbrepatil et al., 2008; Wee and Ryu, 2009). Among the above-mentioned raw materials, lignocellulosic biomass is an inexpensive and widely available renewable carbon source that has no competing food value. Corncob, one of the lignocellulosic biomasses, is an important source of available biomass in the corn-processing industry. More than 10 million tons of it is generated every year in China, and much is left unutilized in harvested fields, causing various environmental problems such as soil and water erosion (Miura et al., 2004; http://www.fao.org; http://www.farmer.com.cn). In China, a part of corncob is hydrolyzed to produce xylitol. It constitutes a new environmentally compatible and sustainable chemical industry for converting the lignocellulosic residues into higher value products. During xylitol production from corncob, a huge amount of corncob molasses is produced as a waste by-product. Corncob molasses is not a food source and contains high concentrations of mixed sugars (about 60–70% total

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sugars), including xylose, glucose, and arabinose, which have the potential to be exploited and utilized, but the effective utilization of corncob molasses has not been previously reported.

The main components of lignocellulose are cellulose and hemicellulose, which mainly consist of glucose and xylose, respectively (Ilmen et al., 2007). Glucose can be utilized by most lactic acid producing bacteria; therefore, the bioconversion of xylose remains as the limiting step. It has been noted that the lack of a microorganism able to ferment efficiently all sugars released by hydrolysis from lignocellulosic materials is one of the main factors preventing utilization of lignocellulose (Garde et al., 2002). The desirability of organisms which could consume xylose without the help of other microorganisms or enzymes is most commonly associated with lactic acid production from lignocellulosic biomass. Some xylose utilizing lactic acid bacteria, such as *Lactobacillus pentosus* and *Lactobacillus brevis*, are available. However, the phosphoketolase pathway used by these organisms converts two of the five carbons in xylose to acetic acid, limiting yields and increasing the cost of product purification (Okano et al., 2009b).

In the present paper, an encouraging process for the economical L-lactic acid production from the biomass sugars in corncob molasses, a waste by-product in xylitol production from corncob widely available, by a newly isolated xylose utilizing thermophilic *Bacillus* sp. strain was investigated.

2. Methods

2.1. Chemicals

Xylose and corncob molasses with a glucose content of 9% (w/v), xylose content of 45% (w/v), and arabinose content of 14.6% (w/v) were purchased from Longlive Bio-technology Co., Ltd (Shandong, China). All other chemicals were of analytical grade and commercially available.

2.2. Isolation of xylose utilizing strain for L-lactic acid production

Soil samples were collected from various areas, including farmland, gardens and land near milk factories. Approximately 2 g of each was enriched in 50 ml of nutrient liquid medium containing 10 g l^{-1} xylose and 10 g l^{-1} yeast extract (YE) at 50 °C without agitation for 6 h. An aliquot of incubation was plated on nutrient agar medium containing (in g l^{-1}): xylose 50, YE 10, $CaCO_3$ 20, agar 20. After 24 h of static incubation at 50 °C, representative colonies were selected based on colony size and acid production zone. Then the selected colonies were incubated in medium containing (in g l^{-1}): xylose 100, YE 20, $CaCO_3$ 75. After 48 h of incubation at 50 °C without agitation, the yields of L-lactic acid were determined and the best xylose utilizing strain for L-lactic acid production, designated as XZL9, was selected for further study.

2.3. Microorganism and culture condition

Strain XZL9 was maintained on Luria–Bertani (LB) agar slant. The slant was inoculated at 50 °C for 24 h and stored at 4 °C. Stock culture was transferred to fresh LB agar slant every 3–4 weeks.

The medium for inoculation contained 50 g l^{-1} glucose, 10 g l^{-1} YE, and 30 g l^{-1} $CaCO_3$. The seed culture was prepared as follows: a loop of cells from the fully grown slant was inoculated into 20 ml of the above sterile medium in 50-ml conical flasks and incubated for 24 h at 50 °C under static conditions, and then the seed culture was inoculated into 300-ml Erlenmeyer flasks for L-lactic acid production. The inoculum volume was 10% (v/v).

2.4. L-Lactic acid fermentation from glucose and xylose using strain XZL9

The fermentation medium used for studying glucose utilization contained 9.1–182.2 g l^{-1} glucose and 10 g l^{-1} YE. Calcium carbonate was added as 60% (w/w) of glucose to the medium. The fermentation medium for studying xylose utilization contained 9.0–152.0 g l^{-1} xylose and 10 g l^{-1} YE. Calcium carbonate was added as 60% (w/w) of xylose to the medium. The fermentation medium for studying utilization of glucose and xylose mixture contained 10 g l^{-1} YE and 60 g l^{-1} $CaCO_3$. The total amount of both sugars was approximately 80 g l^{-1}, with the different percentages of 100% glucose, 50% glucose/50% xylose, and 100% xylose, respectively. Fermentations were carried out at 50 °C under static conditions in 300-ml Erlenmeyer flasks each containing 100 ml medium. Samples were taken periodically and the concentrations of L-lactic acid, residual glucose, and xylose were determined.

2.5. Batch and fed-batch fermentation from xylose

Batch and fed-batch fermentations were conducted in a 5-l bioreactor (BIOSTAT B, B. Braun Biotech International GmbH, Germany) with 3 l fresh medium at 50 °C under static conditions. The medium contained 10 g l^{-1} YE, and the culture pH was maintained at 5.6–6.0 by $CaCO_3$ present in the medium. In the batch fermentation, 81.3 g l^{-1} xylose was used. In the fed-batch fermentation, when the concentration of total sugars was lower than 30 g l^{-1}, xylose was fed into the bioreactor to maintain the total sugars concentration within the range of 30–70 g l^{-1} during the fermentation. Samples were collected periodically to determine the concentrations of L-lactic acid and residual xylose.

2.6. Utilization of various sugars in corncob molasses

The fermentation medium used for studying the reducing sugars utilization contained 70.7 g l^{-1} corncob molasses, 10 g l^{-1} YE, and 40 g l^{-1} $CaCO_3$. The fermentation medium for studying xylose utilization contained 30 g l^{-1} xylose, 10 g l^{-1} YE, and 18 g l^{-1} $CaCO_3$. The fermentation medium for studying glucose utilization contained 5.8 g l^{-1} glucose, 10 g l^{-1} YE, and 4 g l^{-1} $CaCO_3$. The fermentation medium for studying arabinose utilization contained 13.6 g l^{-1} arabinose, 10 g l^{-1} YE, and 8 g l^{-1} $CaCO_3$. Fermentations were carried out at 50 °C under static conditions in 300-ml Erlenmeyer flasks each containing 100 ml medium. Samples were taken periodically and the concentrations of L-lactic acid, total residual reducing sugars, glucose, and xylose were determined.

2.7. Batch and fed-batch fermentations from corncob molasses

Batch and fed-batch fermentations were conducted in a 5-l bioreactor with 3 l fresh medium at 50 °C under static conditions. The medium contained 10 g l^{-1} YE, and the culture pH was maintained at 5.6–6.0 by $CaCO_3$ present in the medium. In the batch fermentation, 150 g l^{-1} corncob molasses (containing 91.4 g l^{-1} of total reducing sugars) was used. In the fed-batch fermentation, when the concentration of reducing sugars was lower than 60 g l^{-1} (xylose concentration was lower than 30 g l^{-1}), the corncob molasses was fed into the bioreactor to maintain the total reducing sugars concentration within the range of 90–100 g l^{-1}. Samples were collected periodically to determine the concentrations of L-lactic acid, total residual reducing sugars, and xylose.

2.8. Analytical methods

The glucose and L-lactic acid concentrations were measured by SBA-80C biosensor analyzer (Institute of Biology, Shandong Acad-

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emy of Sciences, China), which could provide quick measurement of L-lactic acid and glucose based on the technology of immobilized oxidases. The xylose concentration was determined by xylose assay kits (Nanjing Jiancheng Technology Company Ltd, China). The total concentration of reducing sugars was measured by the dinitrosalicylic acid (DNS) method (Miller, 1959). The cell growth was determined by optical density measurements at 620 nm (OD_{620}) in a spectrophotomer (UNICO Instruments Co., Ltd, Shanghai, China). Samples were diluted with 0.3 mol l^{-1} HCl to dissolve the $CaCO_3$ particles.

3. Results and discussion

3.1. Isolation of strain XZL9 for L-lactic acid production from xylose

Strain XZL9 was a homofermentative L-lactic acid producer, which could utilize xylose as the carbon source to produce L-lactic acid. It was Gram-positive, rod shaped, 3.0 to >5.0 μm length and 0.8–0.9 μm in width. Strain XZL9 was tentatively identified as *Bacillus* species according to its 16S rRNA gene sequence (the GenBank accession number: GU556970). The L-lactic acid production from xylose using *Bacillus* sp. strain XZL9 was compared with those produced by some *Bacillus* species under same conditions. *Bacillus* sp. strain XZL9 had the best ability to produce high concentration of L-lactic acid from xylose.

3.2. Utilization of glucose and xylose by strain XZL9

To investigate the utilization of glucose and xylose by *Bacillus* sp. strain XZL9, different initial concentrations of glucose, xylose, and a mixture of glucose and xylose (1:1) were used for L-lactic acid production. As shown in Fig. 1, when the initial glucose concentration was below 100 g l^{-1}, L-lactic acid concentration increased with the addition of glucose and no limitation of L-lactic acid production was observed. The highest L-lactic acid concentration (98.3 g l^{-1}) was obtained at the glucose concentration of 100 g l^{-1}, and the yield of L-lactic acid was approximately 1.0 g g^{-1}. The result implies that *Bacillus* sp. strain XZL9 could metabolize glucose into only L-lactic acid by the homofermentative pathway through EMP, i.e. almost 2 mol of L-lactic acid were produced per mole of glucose consumed. Increasing glucose concentration resulted in longer lag phases and lower consumption rates. With 145.2 g l^{-1} and 182.2 g l^{-1} of initial glucose, glucose consumption was limited.

The time course of xylose utilization by *Bacillus* sp. strain XZL9 is shown in Fig. 2. No limitation was observed with xylose concentrations of 9.0 g l^{-1}, 40.0 g l^{-1}, and 69.2 g l^{-1} and the highest L-lactic acid concentration (67.7 g l^{-1}) was obtained at the xylose concentration of 69.2 g l^{-1}. Substrate inhibition could also be observed in the fermentation with xylose. Increasing xylose concentration (higher than 100 g l^{-1}) resulted in a lower L-lactic acid concentration and more residual xylose. When the initial xylose concentration was 152.0 g l^{-1}, little xylose was consumed (Fig. 2B) and L-lactic acid was hardly produced (Fig. 2A). It has been proposed that an increase in xylose concentration will result in increased intracellular concentrations of the intermediates, such as fructose 1,6-diphosphate, which inhibits enzyme activity during xylose metabolism (Tanaka et al., 2002). When xylose concentration was below 69.2 g l^{-1}, there was nearly no residual xylose in the medium and the yield of L-lactic acid was close to 1.0 g g^{-1}. In xylose-fermenting bacteria, xylose is firstly converted into xylulose-5-phosphate (X5P). This metabolite is further metabolized through either the pentose phosphate pathway (PPP), or through the phosphoketolase pathway (PKP). For the PKP, xylose is converted to lactic acid and acetic acid (Tanaka et al., 2002), while in the PPP, X5P is converted to fructose-6-phosphate and glycoeraldehyde-3-phosphate. These compounds are further metabolized to lactic acid through Embden-Meyerhof pathway (EMP), and the theoretical value of xylose conversion is 1.0 (Patel et al., 2006). In our study, the conversion of xylose to L-lactic acid was close to its theoretical value, thus the PPP was the main pathway for xylose utilization in *Bacillus* sp. strain XZL9.

The average rate of substrate consumption in media containing glucose (1.67 g l^{-1} h^{-1}) was higher than that in media containing xylose (0.93 g l^{-1} h^{-1}). Compared to glucose, the conversion of xylose requires additional enzymatic steps. Some enzymes are inducible; therefore there is a lag time before the enzymes required for assimilation appear when cells are exposed to xylose (Tanaka et al., 2002). The studies indicated that with xylose as substrate, the

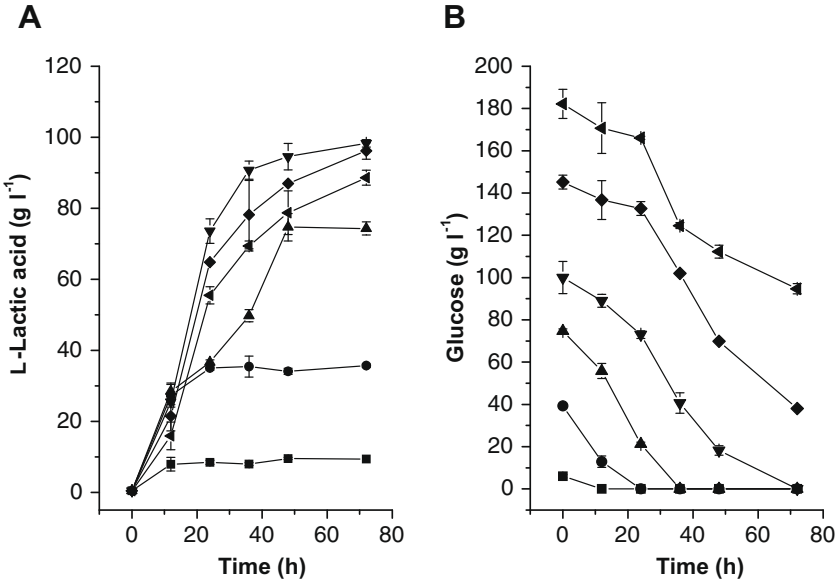

Fig. 1. Time courses of L-lactic acid production and glucose consumption by *Bacillus* sp. strain XZL9. (A) L-lactic acid production. (B) Glucose consumption. Symbols: the initial glucose concentrations (in g l^{-1}) used were at 9.1 (■), 39.3 (●), 74.7 (▲), 100.0 (▼), 145.2 (♦), 182.2 (◄). The error bars in the figure indicate the standard deviations of three parallel replicates. Fermentations were carried out at 50 °C in 300-ml Erlenmeyer flasks containing 100 ml medium under static conditions.

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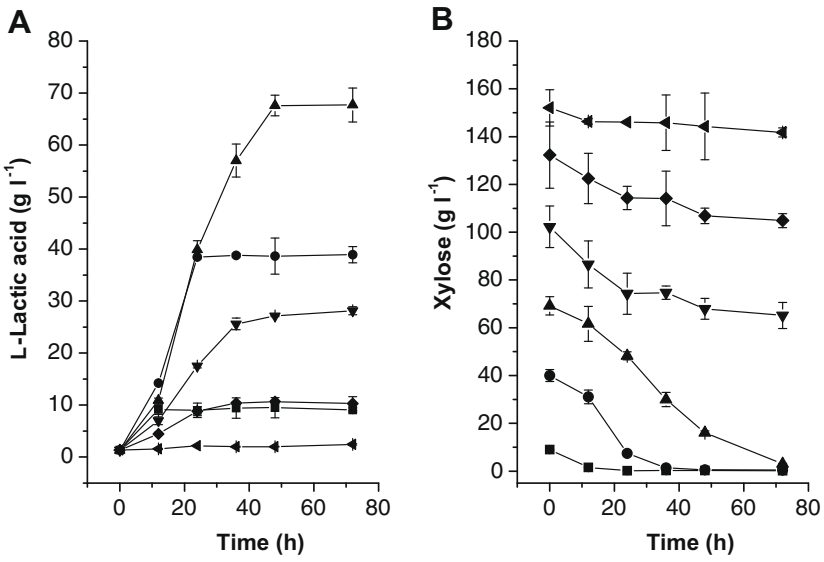

Fig. 2. Time courses of L-lactic acid production and xylose consumption by *Bacillus* sp. strain XZL9. (A) L-lactic acid production. (B) Xylose consumption. Symbols: the initial xylose concentrations (in g l^{-1}) used were at 9.0 (■), 40.0 (●), 69.2 (▲), 102.2 (▼), 132.1 (♦), 152.0 (◄). The error bars in the figure indicate the standard deviations of three parallel replicates. Fermentations were carried out at 50 °C in 300-ml Erlenmeyer flasks containing 100 ml medium under static conditions.

energetically more efficient respiration played a more important role compared to the situation with glucose (Temudo et al., 2009). The higher respiratory flux was related to the complex xylose metabolic pathway.

Studies showed that during the fermentation of lactic acid, the presence of glucose affected the consumption of xylose, which was recognized as the repression of xylose uptake by glucose (Yun and Ryu, 2001). To evaluate the effect of glucose on xylose utilization by *Bacillus* sp. strain XZL9, fermentations were conducted in the medium with glucose or xylose as sole carbon source, and the medium with the combination of glucose and xylose (1:1) as the carbon source. When the initial concentration of glucose, xylose and the sugars mixture was 78.6 g l^{-1}, 69.2 g l^{-1}, and 77.8 g l^{-1}, respectively, same L-lactic acid production was obtained (Fig. 3).

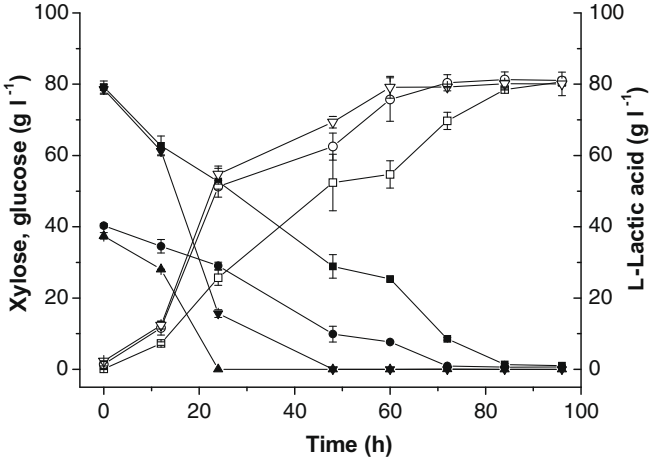

Fig. 3. Time courses of L-lactic acid production and glucose/xylose consumption by *Bacillus* sp. strain XZL9. Symbols: L-lactic acid in the medium with 100% glucose (▽), residual glucose in the medium with 100% glucose (▼), L-lactic acid in the medium with 50% glucose/50% xylose (○), residual glucose (▲) and xylose (●) in the medium with 50% glucose/50% xylose, L-lactic acid in the medium with 100% xylose (□), residual xylose in the medium with 100% xylose (■). The error bars in the figure indicate the standard deviations of three parallel replicates. Fermentations were carried out at 50 °C under static conditions in 300-ml Erlenmeyer flasks containing 100 ml medium.

In the case of L-lactic acid production from the mixture of glucose and xylose (1:1), *Bacillus* sp. strain XZL9 consumed both sugars simultaneously, even though glucose was consumed more rapidly than xylose. The average consumption rate of xylose in media containing 100% xylose (1.09 g l^{-1} h^{-1}) was higher than that in media containing the mixture of glucose and xylose (0.55 g l^{-1} h^{-1}).

Genes required for xylose utilization are repressed in the presence of glucose. This repression is repressed when glucose is almost consumed (Taniguchi et al., 2004). Taking into account the overall utilization of the sugars mixture in lignocellulose, studies about the lactic acid production from both xylose and glucose were carried out. Most microorganisms could not metabolize xylose and glucose simultaneously. Xylose began to be consumed when there was no glucose in the medium (Taniguchi et al., 2004; Ilmen et al., 2007; Okano et al., 2009b). In our study, xylose was slowly consumed by *Bacillus* sp. strain XZL9 in the presence of glucose. The reason may be due to the expression of xylose-catabolizing genes in *Bacillus* sp. strain XZL9 were less repressed by glucose than those in other bacteria (Okano et al., 2009b).

3.3. Batch and fed-batch fermentations from xylose

Batch and fed-batch fermentations were performed in a 5-l bioreactor with initial xylose concentration of 81.3 g l^{-1} and 80.6 g l^{-1}, respectively (Fig. 4). The batch fermentation profile demonstrated two distinct phases (Fig. 4A). The average L-lactic acid productivities of the two phases (0–48 h and 48–144 h) were 1.15 g l^{-1} h^{-1} and 0.46 g l^{-1} h^{-1}, respectively. The production of L-lactic acid terminated at 144 h when the residual xylose was completely consumed and the L-lactic acid titer climbed to its maximum of 80.8 g l^{-1} with a yield of 0.98 g g^{-1}.

In fed-batch fermentation, the L-lactic acid concentration was 115.2 g l^{-1} at 144 h, with a productivity of 0.80 g l^{-1} h^{-1}, which was higher than that of the batch fermentation (Fig. 4B) The highest L-lactic acid concentration (133.8 g l^{-1}) was obtained at 218 h, giving the average productivity of 0.61 g l^{-1} h^{-1}.

On xylose, *Bacillus* sp. strain XZL9 was better at producing L-lactic acid at a greater yield than the best reported strains. Ilmen reported constructed *Pichia stipitis* produced 58 g l^{-1} lactic acid, with a yield of up to 0.58 g g^{-1} xylose (Ilmen et al., 2007). The

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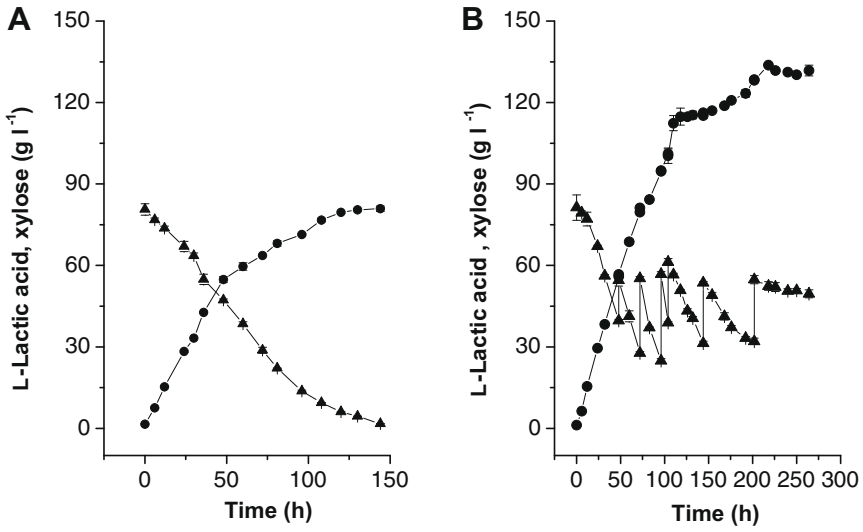

Fig. 4. Time courses of L-lactic acid production from xylose by *Bacillus* sp. strain XZL9 in batch and fed-batch fermentations. (A) Batch fermentation. (B) Fed-batch fermentation. Symbols: L-lactic acid (●), residual xylose (▲). The error bars in the figure indicate the standard deviations of three parallel replicates.

recombinant *Corynebacterium glutamicum* could convert xylose into lactic acid under anaerobic conditions with a yield of 0.54 g g^{-1} (Kawaguchi et al., 2006). From the results obtained in this study, we can conclude that xylose could be efficiently used by *Bacillus* sp. strain XZL9 and high L-lactic acid concentration (133.8 g l^{-1}) was obtained in fed-batch fermentation.

3.4. Utilization of various sugars in corncob molasses

Corncob molasses is a waste and low-cost by-product in the xylitol production from corncob. It contains high concentrations of mixed sugars, including xylose, glucose, and arabinose, which could be used as a carbon source for lactic acid production. Xylose, whose content is 45% (w/v) of the corncob molasses, is the dominant sugar. There have been no previous studies reporting lactic acid production from corncob molasses. In this study, the reducing sugars in corncob molasses could be used by *Bacillus* sp. strain XZL9 to produce L-lactic acid. As shown in Table 1, 26.4 g l^{-1} L-lactic acid was obtained with the yield and productivity of 0.42 g g^{-1} and 0.55 g l^{-1} h^{-1}, respectively. Glucose in corncob molasses was exhausted at 24 h, whereas when the fermentation was terminated at 48 h, the residual xylose and arabinose concentrations in corncob molasses were 11.5 g l^{-1} and 8.93 g l^{-1}, respectively. The consumption rate of xylose, glucose and arabinose was 0.35 g l^{-1} h^{-1}, 0.32 g l^{-1} h^{-1}, and 0.07 g l^{-1} h^{-1}, respectively.

To obtain a better understanding of the utilization of each sugar in the corncob molasses, *Bacillus* sp. strain XZL9 was cultivated on media containing a sole carbon source (xylose, glucose, and arabinose), with the same ratio of concentrations as in corncob molasses. As shown in Table 2, 0.98 g, 0.94 g, and 0.62 g L-lactic acid was produced per gram of xylose, glucose, and arabinose at 48 h, 24 h, and 48 h, respectively. When *Bacillus* sp. strain XZL9 was cultivated in the medium with xylose as carbon source, the conversion of xylose to L-lactic acid was close to its theoretical value (1.0) and there was nearly no residual xylose (0.2 g l^{-1}) in the fermentation medium, while in the case of the fermentation with the sugar mixture, the utilization of xylose was limited and 11.5 g l^{-1} xylose remained in the medium. The result was in accordance with the previous one presented in this study. It has been reported that a single organism had a limited ability to "adjust" its ratio of

Table 1
Fermentation of *Bacillus* sp. strain XZL9 using corncob molasses.*

	Xylose	Glucose	Arabinose
Substrate consumption			
Initial substrate concentration (g l^{-1})	28.4 ± 0.7	7.7 ± 0.2	12.4 ± 1.1
Time of substrate consumption (h)	48	24	48
Concentration of residual substrate (g l^{-1})	11.5 ± 0.3	0	8.9 ± 0.5
Consumption rate of substrate (g l^{-1} h^{-1})[a]	0.35	0.32	0.07
L-lactic acid production			
Time required to reach the maximum concentration of L-lactic acid (h)		48	
Maximum concentration of L-lactic acid (g l^{-1})		26.4 ± 1.0	
L-lactic acid yield (g g^{-1})[b]		0.42	
L-lactic acid productivity (g l^{-1} h^{-1})[c]		0.55	

* Each value is an average of three parallel replicates and is represented as mean ± standard deviation.
[a] [Initial concentration of specific substrate (g l^{-1}) – residual concentration of specific substrate (g l^{-1})]/fermentation time (in h).
[b] g L-lactic acid/g substrate.
[c] Concentration of L-lactic acid (in g l^{-1})/fermentation time (in h).

Table 2
Fermentation of *Bacillus* sp. strain XZL9 using glucose, xylose, and arabinose as the sole carbon source.*

	Xylose	Glucose	Arabinose
Substrate consumption			
Substrate concentration (g l^{-1})	30.0 ± 1.8	5.8 ± 0.1	13.6 ± 0.2
Time of substrate consumption (h)	48	24	48
Concentration of residual substrate (g l^{-1})	0.20 ± 0.03	0	8.27 ± 0.50
Consumption rate of substrate (g l^{-1} h^{-1})[a]	0.62	0.24	0.11
L-lactic acid production			
Maximum concentration of L-lactic acid (g l^{-1})	29.1 ± 0.6	5.4 ± 1.1	3.3 ± 0.2
L-lactic acid yield (g g^{-1}) [b]	0.98	0.94	0.62
L-lactic acid productivity (g l^{-1} h^{-1})[c]	0.61	0.23	0.07

* Each value is an average of three parallel replicates and is represented as mean ± standard deviation.
[a] [Initial concentration of specific substrate (g l^{-1}) – residual concentration of specific substrate (g l^{-1})]/fermentation time (in h).
[b] g L-lactic acid/g substrate.
[c] Concentration of L-lactic acid (in g l^{-1})/fermentation time (in h).

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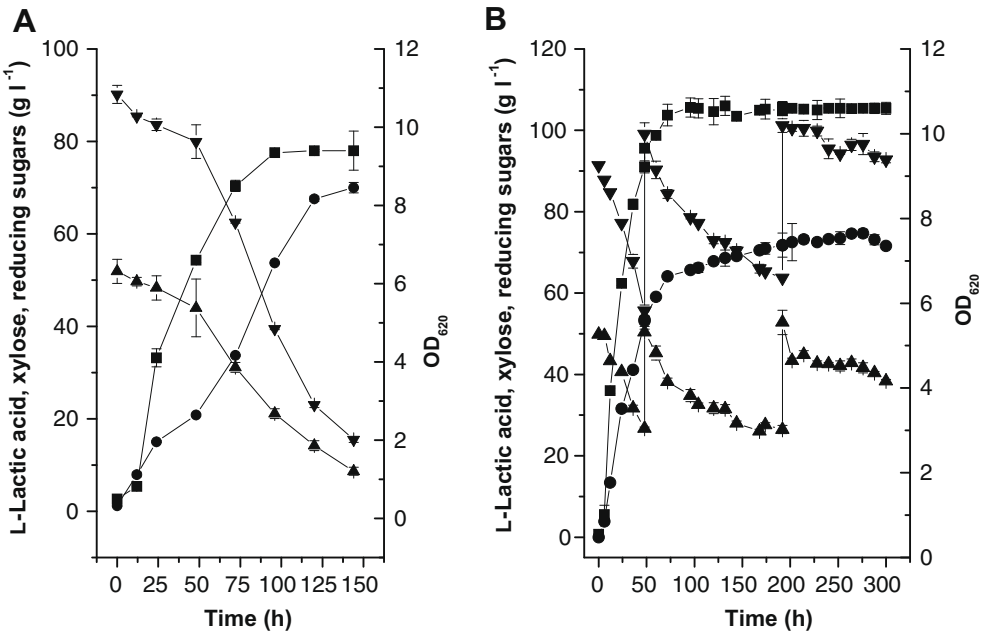

Fig. 5. Time courses of L-lactic acid production from corncob molasses by *Bacillus* sp. strain XZL9 in batch and fed-batch fermentations. (A) Batch fermentation. (B) Fed-batch fermentation. Symbols: L-lactic acid (●), residual xylose (▲), reducing sugars (▼), OD_{620} (■). The error bars in the figure indicate the standard deviations of three parallel replicates.

glucose and xylose consumption rates, and therefore the utilization of a mix of sugars would invariably lead to one of the sugars not being effectively consumed (Eiteman et al., 2009).

In comparison with glucose and xylose, the yield of L-lactic acid (0.62 g g^{-1}) was low during the fermentation with arabinose as the sole carbon source. Arabinose uptake by microorganisms occurs via two distinct systems involving both a low-affinity H^+15-symporter and a high-affinity ATP-dependent transport system. After entering the cell, arabinose is sequentially converted to ribulose, ribulose-5-phosphate, and X5P by the action of arabinose isomerase, ribulokinase, and ribulose-5-phosphate 4-epimerase, respectively. X5P is further converted to lactic acid (Kawaguchi et al.,

Table 3
Comparison of lactic acid production from cellulosic biomass by lactic acid producing bacteria.

Substrate	Organism	Fermentation process	Lactic acid			References
			Concentration (g l^{-1})	Productivity[a] (g l^{-1} h^{-1})	Yield[b] (g g^{-1})	
Alfalfa fibers	*Lactobacillus defbrneckii*	SSF	35.4[e]	0.75	0.35	Sreenath et al. (2001)
	Lactobacillus planlarum	SSF	46.4[e]	0.64	0.46	Sreenath et al. (2001)
Apple pomace	*Lactobacillus rhamnosus* CECT-288	Batch	32.5[c]	5.4	0.88	Gullon et al. (2008)
Barley bran hydrolysates	*Lactobacillus pentosus*	Batch	33.0[e]	0.60	0.57	Moldes et al. (2006)
Cellobiose and cellotriose	*Lactobacillus delbrueckii* Uc-3	Batch	90.0[e]	2.3	0.90	Adsul et al. (2007)
Cellulose	*Lactobacillus delbreuckii* NRRL-B445	SSF	65.0[e]	0.18	–	Iyer and Lee (1999)
Corncob	*Acremonium cellulose* and *Rhizopus* sp.	SSF	24.0[c]	0.17	0.24	Miura et al. (2004)
	Lactobacillus pentosus	Batch	26.0[e]	0.34	0.53	Moldes et al. (2006)
Lignocellulosic hydrolysates	*Lactobacillus* sp. RKY2	Cell–recycle	27.0[e]	6.7	0.90	Wee and Ryu (2009)
Molasses	*Lactobacillus delbrueckii* mutant Uc-3	Batch	166[e]	4.2	0.87	Dumbrepatil et al. (2008)
	Lactobacillus rhamnosus ATCC 7469	SSF	73.0[e]	2.9	0.97	Marques et al. (2008)
Paper sludge	*Bacillus coagulan strains* 36D1	SSCF	92.0[e]	0.96	0.77	Budhavaram and Fan (2009)
	Bacillus coagulan strains P4–102B	SSCF	91.7[e]	0.82	0.78	Budhavaram and Fan (2009)
Rice bran	*Lactobacillus delbrueckii* IFO 3202	SSF	28.0[d]	0.78	0.28	Tanaka et al. (2006)
Sugar cane bagasse	*Bacillus* sp.	Batch	55.5[c]	0.39	0.77	Patel et al. (2004)
	Lactococcus lactis IO-1	Batch	10.9[e]	0.17	0.36	Laopaiboon et al. (2010)
Trimming vine shoots	*Lactobacillus pentosus*	Batch	24.0[e]	0.51	0.76	Moldes et al. (2006)
Wastepaper	*Lactobacillus delbrueckii* B445	SSF	31.0[e]	–	–	Schmidt and Padukone (1997)
Wheat bran hydrolysate	*Lactobacillus bifermentans*	Batch	62.8[e]	1.2	0.83	Givry et al. (2008)
Wood hydrolysate	*Enterococcus faecalis* RKY1	Batch	93.0[c]	1.7	0.93	Wee et al. (2004)
Xylose	Recombinant *Lactobacillus plantarum*	Batch	41.2[d]	–	0.82	Okano et al. (2009b)
Corncob molasses	*Bacillus* sp. strain	Fed batch	74.7[c]	0.38	0.50	Present study

SSF: Simultaneous saccharification and fermentation.
SSCF: Semi-continuous simultaneous saccharification and co-fermentation.
[a] Concentration of L-lactic acid (in g l^{-1})/fermentation time (in h).
[b] Lactic acid (g)/substrate (g).
[c] L-lactic acid.
[d] D-lactic acid.
[e] DL-lactic acid.

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2009; Okano et al., 2009a). In our study, the low consumption rate of arabinose was probably due to deficiencies in arabinose transport (Kawaguchi et al., 2009).

3.5. Batch and fed-batch fermentations from corncob molasses

Bacillus sp. strain XZL9 was cultivated using 25–250 g l^{-1} of corncob molasses (equivalent to 20.49–130.48 g l^{-1} of initial reducing sugars) to investigate the initial concentrations of corncob molasses on L-lactic acid production. The highest L-lactic acid concentration was obtained when the initial corncob molasses concentration was 150 g l^{-1} and a sharp decrease in L-lactic acid production was observed when the corncob molasses concentration was higher than 150 g l^{-1} (data not shown). Batch and fed-batch fermentation were carried out in a 5-l bioreactor with 150 g l^{-1} corncob molasses (initial total reducing sugars concentration of 91.36 g l^{-1}), 10 g l^{-1} YE, and 90 g l^{-1} $CaCO_3$. During batch fermentation, the L-lactic acid concentration increased with an increase in fermentation time (Fig. 5A). The consumption profile of xylose was similar to that of the reducing sugars. After 144 h of fermentation, 70.0 g l^{-1} L-lactic acid was produced. During the first 72 h of fermentation, the cell growth increased rapidly. The highest cell growth (OD_{620} = 9.4) was obtained after 96 h of fermentation.

The profile in fed-batch fermentation could be divided into two parts at the time point of 70 h (Fig. 5B). Prior to 70 h, 70.5 g l^{-1} L-lactic acid was produced, with an average productivity of 1.01 g l^{-1} h^{-1}, which was higher than that of batch fermentation (0.49 g l^{-1} h^{-1}). From 70 h to 300 h, L-lactic acid concentration reached at a value higher than 71.0 g l^{-1}. At the end of the fed-batch fermentation, 74.7 g l^{-1} of L-lactic acid was obtained. Lactic acid production depended strictly on cell growth, and batch fermentation resulted in low cell growth because of the inhibitions of substrate and end-product (Wee and Ryu, 2009). In fed-batch fermentation, the cell growth (OD_{620} = 10.6) was higher than that in batch fermentation (OD_{620} = 9.4). This may be due to the high osmotic pressure of the cells in the batch culture condition.

As the increasing interest in producing biotechnological products from low-cost and renewable biomass, the production of lactic acid from various by-products or agricultural residues has gained considerable interest recently. Many microorganisms, including the fungal species and lactic acid bacteria (LAB), have been investigated for production of lactic acid. However, the high oxygen requirement is the main limitation associated with the fungal species and some examples of microbial lactic acid production from renewable substrates by LAB are shown in Table 3. Relatively low lactic acid concentrations were obtained when corncob (Miura et al., 2004; Moldes et al., 2006), rice bran (Tanaka et al., 2006), and trimming vine shoots (Moldes et al., 2006) were used for lactic acid production. Although a higher concentration of lactic acid was reported using cellobiose and cellotriose (Adsul et al., 2007), molasses (Dumbrepatil et al., 2008), and paper sludge (Budhavaram and Fan, 2009), the mixture of L- and D-lactic acid was produced, which reduced its applicability. In comparison with these renewable substrates, a relatively high concentration of L-lactic acid (74.7 g l^{-1}) was produced from corncob molasses, demonstrating its feasibility as a raw material for L-lactic acid production.

4. Conclusion

As a non-grain raw material, corncob does not compete with grain as an agricultural crop, and the use of corncob molasses has no impact on the food chain for humans. In the present study, we took L-lactic acid as an example for bulk chemical production from corncob molasses, a waste and cheap by-product in the xylitol production from corncob widely available. It may provide an economic L-lactic acid production process with cheap and renewable biomass.

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