J Ind Microbiol Biotechnol (2010) 37:1137–1143
DOI 10.1007/s10295-010-0761-2

ORIGINAL PAPER

Efficient production of lactic acid from sucrose and corncob hydrolysate by a newly isolated *Rhizopus oryzae* GY18

Yang Guo · Qiaojuan Yan · Zhengqiang Jiang · Chao Teng · Xinlei Wang

Received: 5 April 2010 / Accepted: 1 June 2010 / Published online: 16 June 2010

Abstract The aim of this study is to investigate production of L-lactic acid from sucrose and corncob hydrolysate by the newly isolated *R. oryzae* GY18. *R. oryzae* GY18 was capable of utilizing sucrose as a sole source, producing 97.5 g l^{-1} L-lactic acid from 120 g l^{-1} sucrose. In addition, the strain was also efficiently able to utilize glucose and/or xylose to produce high yields of L-lactic acid. It was capable of producing up to 115 and 54.2 g l^{-1} lactic acid with yields of up to 0.81 g g^{-1} glucose and 0.90 g g^{-1} xylose, respectively. Corncob hydrolysates obtained by dilute acid hydrolysis and enzymatic hydrolysis of the cellulose-enriched residue were used for lactic acid production by *R. oryzae* GY18. A yield of 355 g lactic acid per kg corncobs was obtained after 72 h incubation. Therefore, sucrose and corncobs could serve as potential sources of raw materials for efficient production of lactic acid by *R. oryzae* GY18.

Keywords Corncobs · L-Lactic acid · *Rhizopus oryzae* · Sucrose · Xylose

Introduction

Lactic acid (2-hydroxypropanoic acid) is one of the most widely occurring hydroxycarboxylic acids, having diverse applications in food, pharmaceutical, leather, cosmetic, chemical and textile industries and in synthesis of biodegradable plastics [3, 6, 11]. Currently, the most important application of lactic acid is in the production of raw material for the synthesis of the biodegradable polymer polylactic acid, which can solve one of the worldwide environmental problems [15, 27].

Lactic acid can be manufactured either by chemical synthesis or by microbial fermentation. Microbial production of optically pure lactic acid has been extensively studied, because chemically synthesized lactic acid is racemic [22, 34]. Lactic acid can be commercially produced by both bacterial and fungal fermentation [27]. Generally, most of the lactic acid bacteria have higher lactic acid yields and complex nutrient requirements. They ferment sugars via different pathways, resulting in homo-, hetero-, or mixed acid fermentation, though only a few lactic acid bacteria could produce optically pure lactic acid [1, 10, 12, 23, 24]. In addition, some lactic acid bacteria or their mutants can utilize the acid hydrolysate of sucrose for efficient lactic acid production [8, 12, 14]. However, fungi such as *Rhizopus oryzae* have also been proved to be good lactic acid producers. Currently *R. oryzae* is preferable because of its outstanding ability to directly produce almost optically pure L-(+)-lactic acid from glucose or starchy materials with inorganic minerals and ammonium salt as sole nitrogen source [13, 16, 21, 29, 30, 35, 36]. China is one of the largest sucrose-producing countries, producing about 20 million tons, which may be used for lactic acid production. So far, most *Rhizopus oryzae* strains cannot efficiently produce lactic acid from sucrose [4, 33, 35]. Due to the forthcoming scarcity of fossil fuels, lignocellulosic biomass is considered to be an economically attractive carbohydrate feedstock for large-scale fermentation of bulk chemicals such as lactic acid [4, 11, 17, 18, 34]. Corncobs,

Y. Guo · Z. Jiang (✉) · C. Teng · X. Wang
Department of Biotechnology, College of Food Science and Nutritional Engineering, China Agricultural University, No. 17 Qinghua Donglu, Haidian District, PO Box 294, Beijing 100083, China
e-mail: zhqjiang@cau.edu.cn

Q. Yan
Bioresource Utilization Laboratory, College of Engineering, China Agricultural University, Beijing 100083, China

an agricultural waste, however, are mainly used as fuel in furnaces or as manure in soil. More than 30 million tons of corncobs are produced annually in China. Owing to their chemical composition, corncobs show great potential as a renewable raw material for producing a variety of added-value chemicals such as lactic acid, citric acid, xylitol and ethanol [2, 5, 7, 9, 26].

To our knowledge, though lactic acid production from corncobs has been reported, there have been few studies on lactic acid production from simultaneous utilization of hemicelluloses and cellulose in corncobs [2, 20, 26]. In these studies, cellulose was mainly hydrolysed by cellulase to act as carbon source for lactic acid production. Both hemicellulose and cellulose can be hydrolysed into fermentable monosaccharides such as glucose and xylose [25]. Most work deals with simultaneous saccharification and fermentation (SSF) production of lactic acid from hemicellulose-free corncobs in media containing cheap nutrients, and cellulolytic enzymes [20, 25]. Under optimal conditions, *R. oryzae* NRRL-395 yielded 299.4 g lactic acid per kg dry matter of corncobs after 48 h of fermentation at 30°C [26]. Production of L-lactic acid by SSF was also carried out in 3-l airlift bioreactor using *Acremonium thermophilus* and *Rhizopus* sp. MK-96-1196. More than 24 g l^{-1} L-lactic acid was produced from 100 g l^{-1} untreated raw corncobs [20]. Usually, product yields are low without conversion of xylose. To exploit corncobs, whose main monosaccharide constituents are glucose and xylose, fermenting strains must be able to consume both of these sugars and thus to use a larger fraction of the raw material, with correspondingly better yields, than is possible if only glucose is consumed. According to recent reports, several mutants of *R. oryzae* can utilize xylose for efficient production of lactic acid [2, 32]. *R. oryzae* GY18 is a newly isolated strain with the capability of producing L-lactic acid using sucrose and xylose. The objective of this study is to investigate production of L-lactic acid from sucrose and corncob hydrolysate by this strain, which significantly enhanced production of L-lactic acid.

Materials and methods

Materials

Corncob powders (40 mesh) with 34.3% cellulose, 32.5% xylan, 8.9% moisture and 3.1% ash were provided by Shangdong Long-life Co. Ltd. (Yucheng City, Shandong Province, China). Carboxymethylcellulose (CMC, low viscosity) was obtained from Sigma. All other chemicals used were analytical-grade reagents unless otherwise stated.

Microorganism, media and culture conditions

Rhizopus oryzae GY18, an L-(+)-lactic-acid-producing strain selected from soil samples from Shanxi Province, was used in this investigation. The strain was identified by the Institute of Microbiology of Chinese Academy of Sciences (IMCAS) and was deposited (under number CGMCC no. 2681) at the China General Microbiological Culture Collection Center. The strain was maintained on potato dextrose agar (PDA) slants. It was incubated at 35°C. After growth and sporulation, 10 ml distilled water was aseptically added to each agar plate, which was then scraped to release spores. This spore suspension was centrifuged at 4,000 rpm for 10 min; the spores were washed and resuspended in 1 ml distilled water. Then, 1 ml spore suspension containing about 10^6 spores ml^{-1} was used to provide spore inoculum for each 500-ml shake flask containing 100 ml of the medium. Flasks were incubated in a rotary shaker at 35°C and 180 rpm.

To investigate the sucrose tolerance of *R. oryzae* GY18, different initial sucrose concentrations (40–130 g) were used as the principal carbon source in a medium containing (per litre): NH_4Cl 2 g, KH_2PO_4 0.3 g, $MgSO_4{\cdot}7H_2O$ 0.25 g and $ZnSO_4{\cdot}7H_2O$ 0.08 g. After 24 h of inoculation, 70 g l^{-1} sterile $CaCO_3$ in powder form was added to each flask to avoid pH decrease due to lactic acid production [4]. Cultivation was performed at 35°C in 500-ml flasks for 96 h.

L-Lactic acid production from the production media containing glucose only, xylose only or the mixture of glucose and xylose was investigated using 500-ml shake flasks. The fermentation medium contained (per litre of distilled water): glucose (60–180 g) or xylose (20–100 g), and the same amount of salts and $CaCO_3$ as above. Cultivation was performed at 35°C in 500-ml flasks for 72 or 120 h. The results are the average of three fermentations.

Hydrolysate preparation of corncobs

Commercial cellulase was obtained from Shandong Longda Biotechnology Co., Ltd. (Linyi City, Shandong Province, China). The activity of carboxymethyl cellulase and xylanase was 4,000 and 2,600 U g^{-1}, respectively. In order to determine the activity of cellulase and xylanase, the reducing sugar was determined using a 3,5-dinitrosalicylic acid (DNS) colorimetric assay method [19]. One unit of enzyme activity (U) is the amount of enzyme that forms 1 μmol glucose or xylose per min during the hydrolysis reaction.

The hemicellulosic hydrolysate was prepared by dilute acid treatment: 100 g corncobs was immersed in 1,000 ml 1.25% sulphuric acid (v/v) for 24 h, then it was hydrolysed in an autoclave at 121°C for 2 h. The acidic hydrolysis

solution was filtered into two fractions: solid residues and acid hydrolysate. The acid hydrolysate was deionized and neutralized with $CaCO_3$ to pH 6.5, and the precipitated was removed by filtration. Corncobs were pretreated with 1.25% H_2SO_4 as mentioned above, followed by enzymatic hydrolysis using cellulase. Solid residues (10 g based on dry weight) obtained after acidic hydrolysis were hydrolysed with the cellulase (400 U g^{-1} residue) in a 500-ml flask containing 100 ml 50 mmol l^{-1} citrate buffer (pH 5.5) at 50°C for 24 h on a shaking incubator.

The hydrolysates used for making culture media were filtered through a 0.2-μm membrane before fermentation. The composition of the fermentation medium was 100 ml supernatant of hydrolysate with (per litre): $CaCO_3$ 50 g; NH_4Cl 2 g, KH_2PO_4 0.3 g, $MgSO_4{\cdot}7H_2O$ 0.25 g and $ZnSO_4{\cdot}7H_2O$ 0.08 g. The medium was inoculated with 1×10^4 spores/ml. Cultivation was performed at 35°C in 500-ml flasks for 120 h. All experiments were conducted in triplicate, and results are expressed as mean ± standard deviation.

Analysis method

The fermented broths were centrifuged, and supernatants were analyzed for L-(+)-lactic acid and residual carbohydrate. Lactic acid kit (Cat. No. 022) was obtained from Biosentec (France). Lactic acid was determined by high-performance liquid chromatography (HPLC) using Dikma Diamonsil C_{18} column (4.6 × 250 mm) (Dima Co., Ltd., Orlando, FL) and ultraviolet (UV) detector at 210 nm. The eluent, 5 mmol l^{-1} H_2SO_4, was used at a flow rate of 0.8 ml min^{-1}. Sucrose, glucose and xylose were also analyzed with a Sugar-D Waters column (4.6 × 250 mm) in an HPLC system equipped with a refractive index detector (RID). The column was eluted at 40°C with mobile phase of 75:25 (v/v) acetonitrile–water at flow rate of 1.0 ml min^{-1}. Each analysis was performed in triplicate.

Results

Conversion of sucrose by *R. oryzae* GY18

More than 100 *R. oryzae* strains were isolated from various sources in China. One strain was selected, i.e., *R. oryzae* GY18, a lactic acid producer, after a preliminary test of lactic acid production (data not shown). Furthermore, the effect of sucrose on lactic acid production was investigated using *R. oryzae* GY18 (data not shown). GY18 was cultivated in fermentation medium with different initial concentrations of sucrose ranging from 40 to 130 g l^{-1}. Maximum lactic acid productivity of 1.67 g l^{-1} h^{-1} was obtained with initial sucrose concentration of 100 g l^{-1}. Final lactic acid concentration of 80.1 g l^{-1} was achieved after 48 h of fermentation with 0.89 g lactic acid per g sucrose consumed. Moreover, the maximum specific consumption rate of 100 g l^{-1} sucrose by *R. oryzae* GY18 is 2.15 (1 h^{-1}) when the fermentation time was 12 h. During 96 h of fermentation, 97.5 g l^{-1} of lactic acid was produced from 120 g l^{-1} of sucrose with yield of 81.3% based on the initial sucrose concentration (Fig. 1). The use of sucrose at 130 g l^{-1} resulted in dramatic decrease in lactic acid production (Fig. 1). In addition, the produced lactic acid was used for optical isomer determination by lactate dehydrogenase kit enzyme test. *R. oryzae* GY18 produced L-(+)-lactic acid with 98.5 ± 1.0% purity.

Conversion of glucose and xylose by *R. oryzae* GY18

The capability of *R. oryzae* GY18 to convert different initial glucose and xylose concentrations in synthetic media was tested (Table 1). Lactic acid concentration increased from 46.0 to 115 g l^{-1} with increasing glucose concentration in the fermentation medium up to 160 g l^{-1}. Any further increase in glucose level resulted in a decrease in lactic acid concentration. Final lactic acid concentration of

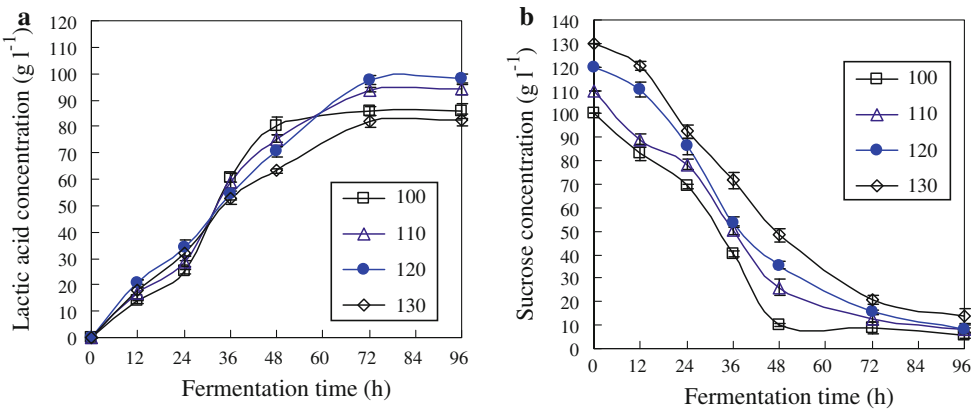

Fig. 1 Lactic acid production (**a**) by *R. oryzae* GY18 and residual sucrose (**b**) in media containing different concentrations of sucrose: 100 g (*open squares*), 110 g (*open triangles*), 120 g (*filled circles*) and 130 g (*open diamonds*). Growth medium was incubated with 1×10^4 spores per millilitre in a rotary shaker with agitation rate of 180 rpm at 35°C

Table 1 Fermentation characteristics of different initial concentrations of glucose and xylose by *R. oryzae* GY18

Initial sugar concentration (g l^{-1})	Lactic acid concentration (g l^{-1})	Lactic acid yield (%)	Residual sugar concentration (g l^{-1})	Biomass (g l^{-1})
Glucose				
60	46.0 ± 1.73	76.6 ± 2.05	0	2.8 ± 0.1
80	68.2 ± 1.82	85.3 ± 2.14	0	3.0 ± 0.15
100	75.2 ± 2.1	82.4 ± 1.32	8.8 ± 0.33	3.6 ± 0.21
120	88.6 ± 2.33	81.2 ± 2.51	10.9 ± 0.28	4.4 ± 0.18
140	103 ± 3.46	81.7 ± 2.32	14.4 ± 0.65	4.3 ± 0.16
160	115 ± 3.27	81.3 ± 1.94	18.2 ± 0.82	4.6 ± 0.22
180	102 ± 2.83	72.9 ± 2.63	40.8 ± 1.33	3.5 ± 0.15
Xylose				
20	14.5 ± 0.21	72.4 ± 1.77	0	3.3 ± 0.1
40	32.2 ± 0.82	80.5 ± 2.45	0	4.2 ± 0.21
60	54.2 ± 1.47	90.2 ± 2.89	0	5.0 ± 0.22
80	59.6 ± 1.89	86.7 ± 1.82	11.3 ± 1.36	5.0 ± 0.21
100	68.5 ± 2.23	84.7 ± 2.25	19.2 ± 1.76	4.8 ± 0.19

Growth medium was incubated with 1×10^4 spores per millilitre in a rotary shaker with agitation rate of 180 rpm at 35°C for 72 h (glucose medium) or 120 h (xylose medium)

115 g l^{-1} was produced by *R. oryzae* GY18 from 160 g l^{-1} glucose after 3 days. This showed a lactic acid yield of 0.81 g g^{-1} glucose consumed. It is generally known that xylose is poorly metabolized by *R. oryzae* compared with glucose. To identify the fermentation capacity of *R. oryzae* GY18 using xylose, the effect of initial xylose concentration on production of lactic acid was also investigated (Table 1). The maximum lactic acid concentration obtained was 54.2 g l^{-1} starting with 60 g l^{-1} xylose. The lactic acid yield was 0.90 g g^{-1} by weight based on the amount of xylose consumed. However, starting with 80 g l^{-1} xylose, it produced 59.6 g l^{-1} of lactic acid with yield of 86.7%.

To study the effect of sugar mixtures on lactic acid production by *R. oryzae* GY18, synthetic media with glucose and/or xylose were used. Beginning with 80 g l^{-1} total sugar (glucose:xylose, 40:40), GY18 produced 65.5 g l^{-1} after 72 h of fermentation with yield of 81.3% (data not shown). Also, the strain showed consumption profiles where glucose was first depleted followed by xylose (Fig. 2). GY18 consumed glucose and xylose completely and produced lactic acid at 42 g l^{-1} after 96 h fermentation. Available glucose (40 g l^{-1}) was consumed completely after 24 h of fermentation, and the maximum concentration was 30 g l^{-1} with productivity of 1.25 g l^{-1} h^{-1}. Consumption of glucose began after incubation, and consumption of xylose started after depletion of glucose. Lactic acid productivity during glucose consumption was 1.25 g l^{-1} h^{-1} and during xylose consumption was 0.20 g l^{-1} h^{-1}. Consumption of xylose started after approximately 36 h. After 96 h of incubation, 20 g l^{-1} of xylose in the culture was completely depleted.

Conversion of corncob hydrolysate by *R. oryzae* GY18

Rhizopus oryzae GY18 has the advantage of being able to convert both glucose and xylose to lactic acid. Corncob hydrolysates contain mainly mixtures of glucose and xylose. Acid hydrolysis liquor of corncobs contained 233 g xylose per kg corncobs and 39.0 g glucose per kg corncobs (Table 2). Enzymatic hydrolysis of solid residues was carried out using the commercial cellulase. The resulting enzymatic hydrolysate was centrifuged, and the supernatant mainly contained 331 g glucose per kg corncobs and 21.1 g xylose per kg corncobs. Finally, 625 g reducing sugars were hydrolysed from 1,000 g corncobs to 370 g glucose and 255 g xylose based on dried corncobs (Table 2). Enhanced production of lactic acid from corncob hydrolysate was achieved by using *R. oryzae* GY18. Furthermore, 205 and 156 g lactic acid was produced from enzymatic hydrolysates and acidic hydrolysates of corncobs, respectively. As shown in Table 2, corncob hydrolysate as carbon source improved lactic acid more than 1.5-fold compared with enzyme hydrolysate. In total, 355 g lactic acid was obtained from 1,000 g corncob hydrolysate. These advantages may offset the disadvantages of using cellulose in corncobs, and the overall lactic acid weight yield will increase.

Discussion

The filamentous fungus *R. oryzae* is an excellent microbial producer of L-(+)-lactic acid from glucose. Comparison of lactic acid production by different *R. oryzae* strains is presented in Table 3. Production levels of lactic

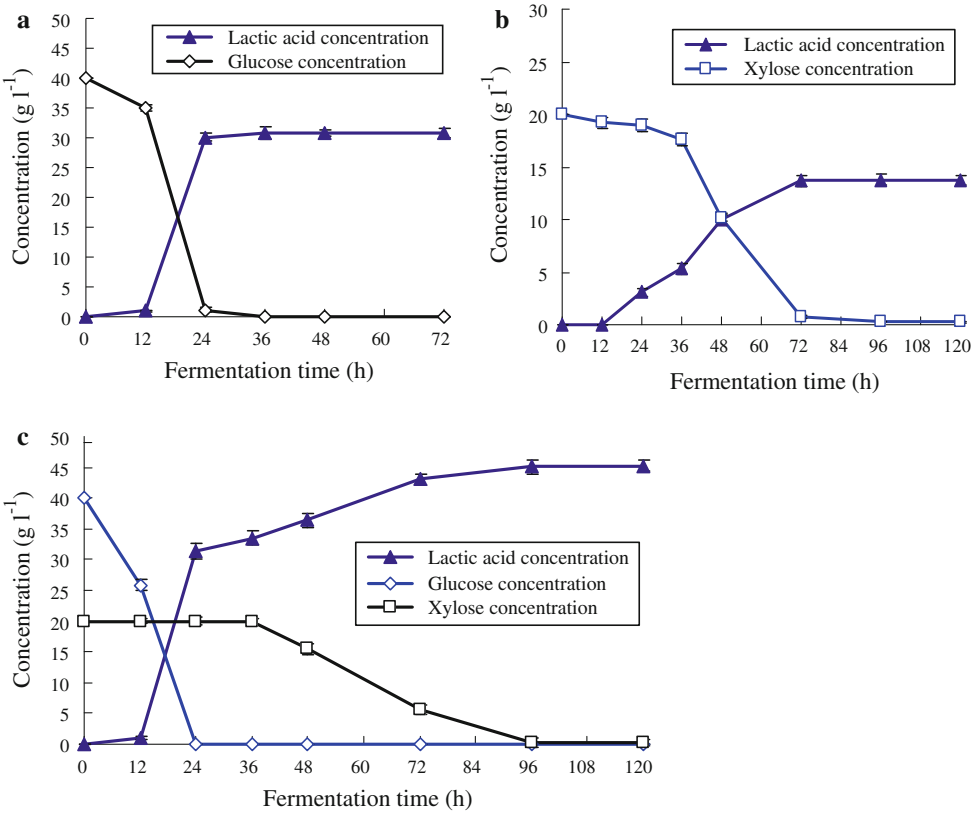

Fig. 2 Lactic acid production by *R. oryzae* GY18 and residual sugars in media containing 40 g l^{-1} glucose (**a**), 20 g l^{-1} xylose (**b**) or 40 g l^{-1} glucose plus 20 g l^{-1} xylose (**c**). Growth medium was incubated with 1×10^4 spores per millilitre in a rotary shaker with agitation rate of 180 rpm at 35°C. *Symbols*: *filled triangles*, lactic acid; *open diamonds*, glucose; *open squares*, xylose

Table 2 Composition of hydrolysates of corncobs and fermentation characteristics by *R. oryzae* GY18

Hydrolysates	Glucose (g kg^{-1} corncobs)	Xylose (g kg^{-1} corncobs)	Lactic acid (g kg^{-1} corncobs)	Lactic acid yield (%)
Enzymatic hydrolysate of corncobs	331 ± 8.2	21.1 ± 1.0	205 ± 7.5	58.4 ± 2.26
Acidic hydrolysate of corncobs	39.0 ± 1.5	233 ± 6.7	157 ± 5.8	57.4 ± 1.79
Hydrolysates of corncobs	370 ± 17.2	255 ± 12.9	355 ± 13.0	58.2 ± 2.33

Growth medium was incubated with 1×10^4 spores per millilitre in a rotary shaker with agitation rate of 180 rpm at 35°C for 72 h

acid vary significantly among the *Rhizopus* species and depend on the carbon sources. Sucrose and corncobs are considered as potentially attractive substrates for production of lactic acid. Our results revealed that the newly isolated *R. oryzae* GY18 is suitable for efficient production of lactic acid using sucrose as carbon source. Lactic acid concentration of 97.5 g l^{-1} with volumetric productivity of 1.35 g l^{-1} h^{-1} was obtained after 72 h of fermentation beginning with 120 g l^{-1} sucrose (Fig. 1). It is known that sucrose is poorly metabolized by most *R. oryzae* strains. There are few reports of *R. oryzae* strains being used for lactic production from sucrose [4, 14, 33]. Production of L-(+)-lactic acid by *R. oryzae* NRRL 395 using sucrose as carbon source has been reported; however, its yield and conversion rate were poor compared with that of using glucose as carbon source. The maximum lactic acid concentration (21 g l^{-1}) was recorded at 50 g l^{-1} sucrose [4]. Among 26 strains of *R. oryzae* cultured in medium containing 100 g l^{-1} sucrose as principal carbon source for 10 days, only two *R. oryzae* strains (NBRC 4789 and 5418) produced lactic acid to some extent (up to 77.3 g l^{-1}) [33]. Comparatively, a sucrose-tolerant *Lactobacillus* sp. strain FCP2, which was grown on sugar-cane juice (mainly 125 g sucrose per litre) for 5 days produced 104 g l^{-1} lactic acid with 90% yield [31].

Generally, *R. oryzae* produces mainly lactic acid from glucose with yields of 60–80% (Table 3). Of 19 *Rhizopus* sp. strains, *Rhizopus oryzae* NRRL 395 produced the highest concentration (65 g l^{-1}) of lactic acid [30]. The highest lactic acid concentration, with yield of 60%, was recorded for *R. oryzae* NRRL 395 when 150 g l^{-1} glucose was present in the medium as sole carbon source [4]. A number of studies have reported improved yields of

Table 3 Comparison of lactic acid production by different *R. oryzae* strains

Rhizopus oryzae	Carbon source (g l^{-1})	Fermentation time (h)	Yield (g l^{-1})	Productivity (g l^{-1} h^{-1})	Reference
GY 18	Sucrose (100)	48	80.1	1.67	This work
	Glucose (160)	72	115	1.60	
	Xylose (100)	120	68.5	0.57	
HZS6	Xylose (80)	90	61.1	0.68	2
NRRL 395	Sucrose (50)	72	21	0.29	4
ADM47.26[a]	Glucose (110)	24	57.9	2.41	13
CBS 112.07	Glucose (120)	72	85.9	1.19	18
	Xylose (58.5)	96	15.2	0.16	
IFO4707	Glucose (50)	72	26.6	0.37	21
IFO5384	Glucose (50)	72	27.7	0.38	21
ATCC 9396	Glucose (125)	40	109	2.73	29
NBRC4785	Sucrose (100)	240	77.8	0.32	31
RQ4015	Glucose (150)	36	121	3.36	32
	Xylose (100)	72	74	1.02	
NRRL 395	Sucrose (120)	72	52.4	0.73	35
	Xylose (120)	72	3.1	0.04	
ATCC 52311	Glucose (94)	32	83.0	2.58	36

[a] Cultures in shaker flasks were first grown at 34°C, with shaking at 120 rpm for 12 h and then the temperature was shifted to 42°C, and maintained at 42°C for another 12 h

85–88% [11, 34, 36]. Furthermore, *R. oryzae* also converts xylose mainly into lactic acid but at a lower rate and with lower yield. Conversion of xylose into lactic acid in synthetic media by ten wild-type *R. oryzae* strains resulted in yields between 0.41 and 0.71 g g^{-1} [17]. When cultures of *R. oryzae* CBS 112.07 were exposed to relatively high xylose concentrations (60–120 g l^{-1}), only 40 g l^{-1} xylose was converted mainly to lactic acid [17]. Two other research groups have reported that *R. oryzae* strains could improve consumption of xylose for production of lactic acid after mutation [2, 32]. An adapted *R. oryzae* HZS6 consumed a mixture of 40 g l^{-1} glucose and 40 g l^{-1} xylose completely and produced lactic acid at 65 g l^{-1} after 66 h fermentation [2]. With the proper energy and dose of ion beam, the mutant RQ4015 of *R. oryzae* PW352 was screened as a promising microbial producer which effectively converted glucose and xylose into lactic acid. When mixed xylose (25 g l^{-1}) and glucose (75 g l^{-1}) were present in a bubble column, L-lactic acid production by the mutant (RQ4015) reached 83 g [32]. In this study, lactic acid yields were 82.4% of 100 g glucose consumed and 90.2% of 60 g xylose consumed.

Corncobs, which contain hemicellulose and cellulose, represent an abundant renewable resource. Use of dilute acids to catalyze hydrolysis of hemicellulose in corncobs to its monosaccharides is well known and effective [7]. As hemicellulose in corncob has been hydrolysed to produce xylose, the corncob residue is porous and can be easily hydrolysed by cellulase [28]. In the present study, corncob was pre-treated by acid hydrolysis followed by enzymatic hydrolysis, which resulted in a hydrolysate that contained a mixture of different monosaccharides released from hemicellulose and cellulose. Glucose and xylose are the main monosaccharides present in corncob hydrolysate. Strain GY18 had efficient ability to utilize both glucose and xylose. Its lactic acid yield was 355 g kg^{-1} dry weight corncobs. Our results show that production of L-lactic acid obtained by simultaneous utilization of hemicelluloses and celluloses in corncobs is feasible and efficient. Using corncob as a substrate, a yield of 299 g lactic acid per kg dry matter of corncobs was reported using *R. oryzae* NRRL-395 [26]. Similarly, an adapted *R. oryzae* strain HZS6 that significantly improved efficiency of substrate utilization and enhanced production of L-(+)-lactic acid from corncob hydrolysate. It increased L-(+)-lactic acid final concentration, yield, and volumetric productivity more than twofold compared with its parental strain [2].

Conclusions

Rhizopus oryzae GY18 was found to be a promising strain which could rapidly ferment sucrose to lactic acid and convert glucose/xylose into lactic acid with high yield and productivity. It can render bioconversion of corncobs to lactic acid more attractive due to increased final lactic acid production.

Acknowledgments This work was financially supported by the National Basic Research Program of China (973 Program No.

2007CB714303) and New Century Excellent Talents in University (NCET-08-0534).

References

1. Altaf M, Venkateshwar M, Srijana M, Reddy G (2007) An economic approach for L-(+) lactic acid fermentation by *Lactobacillus amylophilus* GV6 using inexpensive carbon and nitrogen sources. J Appl Microbiol 103(2):372–380
2. Bai DM, Li SZ, Liu ZL, Cui ZF (2008) Enhanced L-(+)-lactic acid production by an adapted strain of *Rhizopus oryzae* using corncob hydrolysate. Appl Biochem Biotechnol 144(1):79–85
3. Brinques GB, do Carmo Peralba M, Ayub MA (2010) Optimization of probiotic and lactic acid production by *Lactobacillus plantarum* in submerged bioreactor systems. J Ind Microbiol Biotechnol 37(2):205–212
4. Bulut S, Elibol M, Ozer D (2004) Effect of different carbon sources on L(+)-lactic acid production by *Rhizopus oryzae*. Biochem Eng J 21(1):33–37
5. Chen M, Xia LM, Xue PJ (2007) Enzymatic hydrolysis of corncob and ethanol production from cellulosic hydrolysate. Int Biodeterior Biodegrad 59(2):85–89
6. Datta R, Tsai SP, Bonsignore P, Moon SH (1995) Technological and economic potential of poly(lactic acid) and lactic acid derivatives. FEMS Microbiol Rev 16:221–231
7. Dominguez JM, Cao N, Gong CS, Tsao GT (1997) Dilute acid hemicellulose hydrolysates from corn cobs for xylitol production by yeast. Bioresour Technol 61(1):85–90
8. Dumbrepatil A, Adsul M, Chaudhari S, Khire J, Gokhale D (2008) Utilization of molasses sugar for lactic acid production by *Lactobacillus delbrueckii* subsp. *delbrueckii* mutant Uc-3 in batch fermentation. Appl Environ Microbiol 74(1):333–335
9. Hang YD, Woodams EE (2001) Enzymatic enhancement of citric acid production by *Aspergillus niger* from corn cobs. Lebensm-Wiss Technol 34(7):484–486
10. Hofvendahl K, Hahn-Hagerdal B (2000) Factors affecting the fermentative lactic acid production from renewable resources (1). Enzyme Microb Technol 26:87–107
11. John RP, Nampoothiri KM, Pandey A (2007) Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives. Appl Microbiol Biotechnol 74(3):524–534
12. Kadam SR, Patil SS, Bastawde KB, Khire JM, Gokhale DV (2006) Strain improvement of *Lactobacillus delbrueckii* NCIM 2365 for lactic acid production. Process Biochem 41(1):120–126
13. Liaw HJ (2003) Novel strains of *Rhizopus oryzae* and uses thereof. US 2003/0003553 A1
14. Lunelli BH, Andrade RR, Atala DI, Wolf Maciel MR, Maugeri Filho F, Maciel Filho R (2010) Production of lactic acid from sucrose: strain selection, fermentation, and kinetic modeling. Appl Biochem Biotechnol 161(1–8):227–237
15. Lunt J (1998) Large-scale production, properties and commercial applications of polylactic acid polymers. Polym Degrad Stab 59:145–152
16. Liu Y, Liao W, Chen S (2008) Co-production of lactic acid and chitin using a pelletized filamentous fungus *Rhizopus oryzae* cultured on cull potatoes and glucose. J Appl Microbiol 105(5): 1521–1528
17. Maas RHW, Bakker RR, Eggink G, Weusthuis RA (2006) Lactic acid production from xylose by the fungus *Rhizopus oryzae*. Appl Microbiol Biotechnol 72(5):861–868
18. Maas RH, Springer J, Eggink G, Weusthuis RA (2008) Xylose metabolism in the fungus *Rhizopus oryzae*: effect of growth and respiration on L(+)-lactic acid production. J Ind Microbiol Biotechnol 35(6):569–578
19. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem 31:426–428
20. Miura S, Arimura T, Itoda N, Dwiarti L, Feng JB, Bin CH, Okabe M (2004) Production of L-lactic acid from corncob. J Biosci Bioeng 97(3):153–157
21. Oda Y, Yajima Y, Kinoshita M, Ohnishi M (2003) Differences of *Rhizopus oryzae* strains in organic acid synthesis and fatty acid composition. Food Microbiol 20(3):371–375
22. Okano K, Tanaka T, Ogino C, Fukuda H, Kondo A (2010) Biotechnological production of enantiomeric pure lactic acid from renewable resources: recent achievements, perspectives, and limits. Appl Microbiol Biotechnol 85(3):413–423
23. Okano K, Zhang Q, Shinkawa S, Yoshida S, Tanaka T, Fukuda H, Kondo A (2009) Efficient production of optically pure D-lactic acid from raw corn starch by using a genetically modified L-lactate dehydrogenase gene-deficient and α-amylase-secreting *Lactobacillus plantarum* strain. Appl Environ Microbiol 75(2): 462–467
24. Petrov K, Urshev Z, Petrova P (2008) L(+)-Lactic acid production from starch by a novel amylolytic *Lactococcus lactis* subsp. *lactis* B84. Food Microbiol 25(4):550–557
25. Rivas B, Moldes AB, Domínguez JM, Parajó JC (2004) Lactic acid production from corn cobs by simultaneous saccharification and fermentation: a mathematical interpretation. Enzyme Microbiol Technol 34(7):627–634
26. Ruengruglikit C, Hang YD (2003) L (+)-Lactic acid production from corncobs by *Rhizopus oryzae* NRRL-395. Lebensm-Wiss Technol 36(6):573–575
27. Sauer M, Porro D, Mattanovich D, Branduardi P (2008) Microbial production of organic acids: expanding the markets. Trends Biotechnol 26(2):100–108
28. Shen X, Xia L (2006) Lactic acid production from cellulosic material by synergetic hydrolysis and fermentation. Appl Biochem Biotechnol 133(3):251–262
29. Sheu DC, Duan KJ, Wu CY, Yu MC (2006) Method for producing L-lactic acid. US 2006/0234361 A1
30. Soccol CR, Stonoga VI, Raimbault M (1994) Production of L-lactic acid by *Rhizopus* species. World J Microbiol Biotechnol 10(4):433–435
31. Timbuntam W, Sriroth K, Tokiwa Y (2006) Lactic acid production from sugar-cane juice by a newly isolated *Lactobacillus* sp. Biotechnol Lett 28(11):811–814
32. Wang P, Li J, Wang L, Tang ML, Yu ZL, Zheng ZM (2009) L (+)-Lactic acid production by co-fermentation of glucose and xylose with *Rhizopus oryzae* obtained by low-energy ion beam irradiation. J Ind Microbiol Biotechnol 36(11):1363–1368
33. Watanabe T, Oda Y (2008) Comparison of sucrose-hydrolyzing enzymes produced by *Rhizopus oryzae* and *Amylomyces rouxii*. Biosci Biotechnol Biochem 72(12):3167–3173
34. Wee YJ, Kim JN, Ryu HW (2006) Biotechnological production of lactic acid. Food Technol Biotechnol 44(2):163–172
35. Yin P, Nishina N, Kosakai Y, Yahiro K, Pakr Y, Okabe M (1997) Enhanced production of L(+)-lactic acid from corn starch in a culture of *Rhizopus oryzae* using an air-lift bioreactor. J Ferment Bioeng 84(3):249–253
36. Zhou Y, Domínguez JM, Cao N, Du J, Tsao GT (1999) Optimization of L-lactic acid production from glucose by *Rhizopus oryzae* ATCC 52311. Appl Biochem Biotechnol 77–79:401–407