RESEARCH ARTICLE

Microbial production of stereospecific lactic acid from sugarcane trash hydrolysate with no pre-detoxification step

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Abstract: Sugarcane trash (SCT) is a promising underused lignocellulosic material for the production of chemicals in biorefineries. In this work, we studied the use of SCT to produce lactic acid (LA), an important commodity chemical used in food and bioplastic industries. SCT was pretreated with a hydrothermal liquid hot water step followed by saccharification with Cellic® CTec2, which resulted in the SCT hydrolysate containing 30.42 ± 0.08 g/L total sugar, equivalent to 88.10% and 42.92% glucose and xylose recoveries, respectively. Screening of stereospecific LA production exhibited *Lactococcus lactis* BCC 68868 and *Leuconostoc lactis* BCC 62792 efficiently utilized SCT hydrolysate converted to entirely L-LA and D-LA, respectively. A detoxification step was unnecessary for hydrolysate fermentation to LA, and the addition of $CaCO_3$ for pH control enabled complete hydrolysate utilization. *La. lactis* BCC 68868 converted SCT hydrolysate into optically pure L-LA at 19.34 g/L, equivalent to a conversion yield of 0.95 g LA/g sugar. While *Le. lactis* BCC 62792 produced D-LA at 10.39 g/L, equivalent to a conversion yield of 0.45 g LA/g sugar with ethanol and acetic acid as the by-products, suggesting their homofermentative and heterofermentative characteristic, respectively. This work demonstrates a promising strategy to produce enantiomerically defined LA from this underutilized agricultural waste.

Keywords: Sugarcane trash; fermentation; lactic acid; lactic acid bacteria; stereospecific

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1 Introduction

Lactic acid (LA) is an organic acid that is a commodity chemical used in food as well as cosmetics, medicine, and chemical industries. Currently, the utilization of LA as the monomer for the production of poly-lactide (PLA) polymers has increased remarkably due to the rising demand for biodegradable plastics (de Albuquerque et al., 2021; Huang et al., 2023b). The physical properties of PLA depend on its D-LA and L-LA composition (Farah et al., 2016;Han et al., 2022). Poly L-lactic acid (PLLA) is a thermostable L-LA-based homopolymer widely used for food packaging, e.g., food containers, blister packaging, and disposable cups (He et al., 2023). Compared to PLLA, poly D-lactic acid (PDLA) is heat sensitive and more biodegradable and used for applications with heat deflection temperatures lower than 55°C such as cutlery, cups, plates, and saucers (Huang et al., 2021). Mixing D-LA and L-LA in different ratios therefore affects the properties and biodegradability of the resulting material (Swetha et al., 2023).

LA can be produced using either chemical synthesis or microbial fermentation. Nevertheless, microbial fermentation has an advantage in its potential to produce specifically pure isomers of LA compared with chemical synthesis which results in a racemic mixture (Ojo and de Smidt, 2023). LA is produced from various microorganisms such as fungi and bacteria. Lactic acid bacteria (LAB) are a kind of Gram-positive microorganisms, catalase-negative that can ferment carbohydrates to produce lactic acid as the major end-product (Abedi and Hashemi, 2020). LABs have a reputation of being generally recognized as safe (GRAS) and are widely applied for food fermentation and use as probiotics or as efficient microbial cell factories (Pontes et al., 2021). LA can be synthesized either in the form of an optically pure LA (D-LA or L-LA) or as a racemic mixture (DL-LA) by the homofermentative and heterofermentative LAB strains (Okano et al., 2010). The specificity of LA enantiomers depends on the bacteriums lactate enzyme, which is responsible for catalyzing pyruvate to distinct LA enantiomers during lactate metabolism. L-lactate dehydrogenase (EC 1.1.1.27)

converts pyruvate to L-lactate while D-lactate dehydrogenase (EC 1.1.1.28) generates D-lactate (Pohanka, 2020).

LA fermentation processes conventionally utilize refined sugar and starch as substrates, constituting a significant proportion of the total production cost. To reduce production costs, the use of alternative raw materials such as agricultural wastes or lignocellulosic biomass, a zero-carbon and renewable feedstock that contributes to the reduction of global warming, has garnered increasing interest (Ahmad et al., 2020; Huang et al., 2023a). However, the use of lignocellulosic material introduces a potential challenge, as the release of toxic by-products during processing can impede the efficiency of the fermentation process. Consequently, a detoxification step is generally required before the fermentation step, which adds cost to the process. Sugarcane trash (SCT) comprises leaves and tops as wastes from the harvesting step. In the past, these parts of sugarcane were burnt in the vicinity of the field blaze before the harvesting step as a general practice, causing the generation of air pollution. With an increase in global awareness, several countries have started to implement a ban on field burning for sugarcane harvesting. Therefore, increasing amounts of SCT are expected, making it a promising raw material for biorefineries (Powar et al., 2022). Nevertheless, the utilization of SCT converted to value-added products has been limited. Most of the study focuses on the usage of sugarcane bagasse, a solid residue from sugar mills. In this study, we report the production of stereospecific LA from SCT by different LAB strains. SCT was pretreated by hydrothermal liquid hot water (LHW) which was a chemical-free pretreatment method that generated low amounts of inhibitory by-products (Chen et al., 2022). In combination with the use of the stereospecific LA-producing strains, this led to the direct fermentation of SCT hydrolysate to LA with no need for a prior detoxification step. The work demonstrates a simple and straightforward process for the production of an optically pure LA from SCT in the biorefinery industry.

2 Materials and Methods

2.1 Pretreatment of SCT

SCT was collected from Phetchabun province, Thailand. It was physically milled using a 20BVI pin mill (Shanghai UPG, China) and sieved to obtain particles with diameters of 0.5-1 mm. Afterward, the biomass was pretreated using liquid hot water (LHW) in a 7.5-L high-pressure reactor (Parr Reactor 4848, Parr Instrument, Moline, IL Company, USA) with a solid:liquid ratio of 1:12 (400 g of SCT: 4.8 L of distilled water). The reaction was operated at 180°C at a heating rate of 1.7°C/min with 400 rpm mixing speed (Egüés et al., 2012). The reaction was immediately quenched after reaching the target temperature and then cooled down. The solid fraction was separated by a mesh filter and washed with distilled water until the pH reached 5.5 and then dried at 70°C overnight before performing enzymatic hydrolysis. The raw SCT composition of cellulose, hemicellulose, lignin, and ash content was analysed by the National Renewable Energy Laboratory (NREL) method (Sluiter et al., 2010).

2.2 Optimization of SCT hydrolysis

Pretreated SCT was saccharified using a commercial enzyme mixture (Cellic® CTec2, Novozymes AS, Bagsvrd, Denmark) with a specific activity of 2.4 Filter Paper Units (FPU)/mg protein (176.4 FPU/ml). Hydrolysis reaction was performed in 1 mL contained 1% (w/v) pretreated biomass with 1.25, 2.5, 5, 10, and 20 mg protein/g substrate in 50 mM sodium acetate buffer, pH 5.0, and incubated at 50°C for 72 h with shaking at 200 rpm. The reaction mixture was stopped by heating at 100°C for 10 min and clarified by centrifugation at 6,797 ×g for 10 min. Liquid fractions were analysed for sugar profiles by high-performance liquid chromatography (HPLC) equipped with a refractive index detector using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) operating at 65°C with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.5 mL/min.

2.3 Preparation of SCT hydrolysate

Biomass hydrolysate (800 mL) was prepared in a 2-L Erlenmeyer flask. The reaction contained 5% (w/v) pretreated SCT with 20 mg protein/g substrate of Cellic® CTec2 (equivalent to 48 FPU/g substrate) in 50 mM sodium acetate buffer pH 5.0. The reaction was incubated at 50°C with shaking at 200 rpm for 72 h before quenching by heating at 100°C for 10 min. After that, the reaction was clarified by centrifugation at 6,797 ×g for 10 min and the collected liquid fraction was filtered through 0.22-μm syringe filters. Sugar, organic acid, and inhibitor profiles were analyzed by HLPC as described above.

2.4 Microorganisms and cultivation

LAB strains in the *Lactobacillus, Lactococcus, Leuconostoc, Levilactobacillus,* and *Weizmannia* genera were provided by the Thailand Bioresource Research Center collection (TBRC) (www.tbrcnetwork.org). Seed cultures were prepared as follows: one loop of cells from stock culture was inoculated into 5 ml of de Man, Rogosa, and Sharpe medium (MRS), comprising 20.0 g/L glucose, 10.0 g/L peptone, 5.0 g/L yeast extract, 10.0 g/L beef extract, 1.0 mL/L Tween 80, 2.0 g/L ammonium citrate, 5.0 g/L sodium acetate, 0.2 g/L $MgSO_4$, 0.05 g/L $MnSO_4$, and 2.0 g/L K_2HPO_4. Cultures were incubated at 30°C with 150 rpm for 18 h.

2.5 Screening of potential LAB

In total, twenty LAB strains from the TBRC collection were screened for their capability to use SCT hydrolysate as a

carbon source. Fermentation (5 mL) was performed in 50-mL screw-capped tubes containing 50% SCT hydrolysate supplemented with nitrogen sources as in MRS medium, including peptone (10.0 g/L), yeast extract (5.0 g/L) and beef extract (10.0 g/L). Cultures were incubated at 30°C under static conditions for 48 h. Cell growth was monitored with a spectrophotometer at 600 nm. Supernatants were collected by centrifugation at 12,396 ×g for 10 min and filtered through 0.22-μm syringe filters. Production of L-LA and D-LA was analyzed by HPLC with a UV detector set at 230 nm and a Shodex CRX853 column (Showa Denko K.K. Tokyo, Japan). A $CuSO_4$ solution (0.5 mM) was used as the mobile phase at a flow rate of 0.25 mL/min for 40 min. The column temperature was maintained at 40°C. The yield of LA (g/g) was defined as the ratio of lactic acid produced (g/L) to the amount of sugar consumed (g/L).

2.6 Production of stereospecific LA

Two LAB candidates for producing L-LA (*La. lactis* BCC 68868) and D-LA (*Le. lactis* BCC 62792) were cultured in 250-mL Erlenmeyer flasks containing 25 mL 100% of SCT hydrolysate supplemented with nitrogen sources including peptone (10.0 g/L), yeast extract (5.0 g/L) and beef extract (10.0 g/L). Cultures were incubated at 30°C under static conditions for 0-72 h. Cell growth was monitored with a spectrophotometer at 600 nm. The stereospecific LA production was detected in supernatants by HPLC with a UV detector set at 230 nm and a Shodex CRX853 column. The fermentation medium was supplemented with 1.0% w/v $CaCO_3$ to study the effect of $CaCO_3$ on LA production.

3 Results and Discussion

3.1 Preparation of SCT hydrolysate

The composition of untreated SCT analyzed in this study, based on dry weight, consisted of cellulose 34.49 ± 0.53%, hemicellulose 29.72 ± 0.68%, lignin 19.58 ± 0.25%, and ash 6.44±0.13% with composition percentages falling in the same range as previous studies (Miftah et al., 2022). The environmentally friendly LHW process, which does not require the use of chemicals and produces fewer inhibitor by-products, was applied in the pretreatment process. After that, enzymatic saccharification was performed to hydrolyze complex lignocellulosic structures. The commercial cellulolytic enzyme Cellic® CTec2 was selected for this process as it comprises complex polysaccharide-degrading activities, including CMCase, FPase, CBH1, β-glucosidase, and β-xylosidase activities. These contribute to synergistic interactions that efficiently hydrolyze lignocellulosic materials (Sun et al., 2015). The saccharification experiment was performed by varying the enzyme dosage from 1.25 to 20 mg/g substrate, equivalent to the enzyme activity of 3 to 48 FPU/g, and incubation time from 24 to 72 h. Concentrations of released glucose and xylose increased along with increasing enzyme dosage and incubation time (Figure 1A and 1B). The highest glucose and xylose yields, at 636.52 and 205.42 mg/g substrate respectively, were obtained from the reaction with an enzyme dosage of 20 mg/g substrate at 72 h. The sugar yields obtained were relatively high compared with previous works using sugarcane bagasse as the substrates with steam explosion pretreatment (dos Reis et al., 2013).

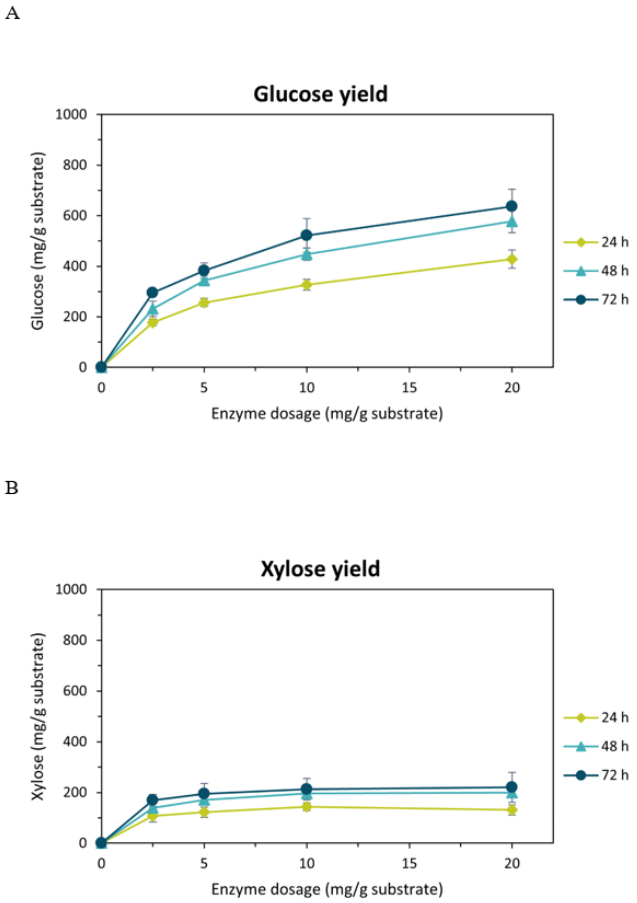

Figure 1. Glucose (A) and xylose (B) products from enzymatic hydrolysis in 50 mM sodium acetate, pH 5.0, of 1% (w/v) LHW pretreated sugarcane trash at 50°Cfor 24, 48 and 72 h.

Using this optimal condition for SCT hydrolysis, we scaled up this enzymatic hydrolysis process to characterize the resulting hydrolysate. SCT hydrolysates were found to contain a total sugar concentration of 30.42 ± 0.08 g/L (21.10 ± 0.03 g/L glucose, 9.06 ± 0.02 g/L xylose and 0.26 ± 0.03 g/L arabinose), which is equivalent to 88.10% glucose recovery and 42.92% xylose recovery based on the glucose and xylose available in the raw SCT. In addition, the hydrolysates also contained organic acids (0.57 ± 0.00 g/L LA, 4.34 ± 0.01 g/L acetic acid, and 0.01 ± 0.00 g/L levulinic acid) generated as by-products from the pretreatment step (Table 1). The presence of hydroxymethylfurfural (HMF) and furfural, which are strong inhibitors of cell growth and fermentation (van der Pol et al., 2016), was not detected under these experimental conditions. Altogether, these results demonstrated that our

developed pretreatment and hydrolysis protocols are suitable for the production of biomass-derived sugars and promising for LA fermentation without requiring a detoxification step.

Table 1. Sugarcane trash composition after liquid hot water pretreatments

Sugars or By-products	Concentration (mg/ml)
Sugars	30.42 ± 0.08
Glucose	21.10 ± 0.03
Xylose	9.06 ± 0.02
Arabinose	0.26 ± 0.03
Total by-products	4.92 ± 0.01
Lactic acid	0.57 ± 0.00
Acetic acid	4.34 ± 0.01
Levulinic acid (LLA)	0.01 ± 0.00
HMF	ND
Furfural	ND

Abbreviations: ND, not detectable. Data presented as mean ± s.d.

3.2 Screening of LAB strains for stereospecific LA production from SCT hydrolysate

Twenty candidate LAB strains were selected from the TBRC culture collection based on literature reports on their capacity of LA production and particularly screened for ability to produce specific LA enantiomers with SCT hydrolysate. Preliminary results using an SCT medium containing 50% SCT hydrolysate supplemented with nitrogen sources revealed that all strains produced LA at varying levels from 0.94 - 12.80 g/L, equivalent to a conversion yield of 0.09 - 0.89 g LA/g sugar (Table 2). All *Lactobacillus pentosus* and *Levilactobacillus brevis* produced mixtures of DL-LA while *La. lactis* and *Weizmannia coagulan* produced optically pure L-LA. Interestingly, only the sole strain of *Le. lactis* produced pure D-LA. As reported previously, the specificity of LA enantiomers of a given LAB is dependent upon the type of lactate dehydrogenase used for lactate metabolism (Pohanka, 2020). The two candidate LAB strains that gave the highest conversion (g LA/g sugar) of stereospecific LA, *La. lactis* BCC 68868, produced 4.01 g/L of L-LA and *Le. lactis* BCC 62792 produced 4.46 g/L of D-LA were selected as candidate strains for further study.

3.3 Stereospecific LA production from 100% SCT hydrolysate

To further investigate whether the candidate LAB strains are capable of directly utilizing the SCT hydrolysate, LA production using the non-detoxified SCT hydrolysate with nitrogen supplements was explored. Results showed that *La. lactis* BCC 68868 converted 29.6% of glucose (6.51 g/L) to L-LA, with L-LA production plateauing after 24 h at a peak of 5.11 ± 0.05 g/L, equivalent to 0.72 g LA/g sugar (Figure 2A). On the other hand, *Le. lactis* BCC 62792 utilized more than 58.2% glucose (12.9 g/L) and 16.1% xylose (1.01 g/L) in the SCT hydrolysate but produced only a low yield of LA. The maximum yield of D-LA was obtained after 36 h at 6.50 ± 0.06 g/L, equivalent to 0.47 g LA/g sugar (Figure 2B). To improve the productivity of LA, optimization methods such as increasing the initial glucose concentration, implementing a detoxification step to remove inhibitors, and eliminating the feedback inhibition effect by pH control were established (Rawoof et al., 2020; Wang et al., 2015). Nevertheless, in this study, growth-inhibitory by-products such as furfural and HMF are absent in SCT hydrolysate during the LHW pretreatment process. To improve stereospecific LA production of *La. lactis* BCC 68868 and *Le. lactis* BCC 62792, the evidence by pH control using $CaCO_3$ was further investigated.

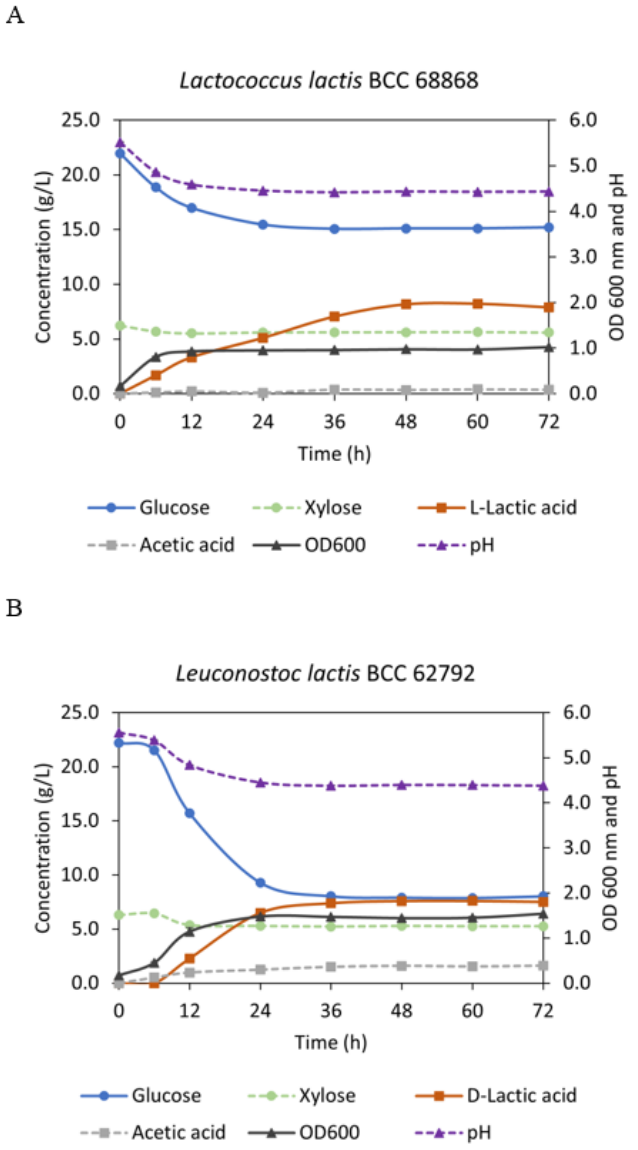

Figure 2. Growth profiles of Lactococcus lactis BCC 68868 (A) and Leuconostoc lactis BCC 62792 (B) grown on SCT hydrolysate supplemented with nitrogen sources.

Table 2. Screening of LAB strains potentially useful for SCT hydrolysate fermentation.

LAB strains	LA titer (g/L)	L-LA titer (g/L)	D-LA titer (g/L)	L:D-LA ratio	Conversion (g LA/g sugar)
Lactobacillus pentosus TBRC 11043	12.80	2.42	10.38	19:81	0.89
Lactobacillus pentosus TBRC 8210	12.53	2.45	10.08	20:80	0.87
Lactobacillus pentosus TBRC 9679	11.79	2.82	8.97	24:76	0.81
Lactobacillus pentosus TBRC 6978	11.32	2.14	9.18	19:81	0.79
Lactobacillus pentosus BCC 42271	12.64	2.67	9.97	21:79	0.85
Lactobacillus pentosus BCC 42471	12.55	3.37	9.18	27:73	0.84
Lactobacillus pentosus TBRC 8579	12.42	3.02	9.40	24:76	0.86
Lactobacillus pentosus BCC 42573	12.36	2.98	9.38	24:76	0.85
Lactococcus lactis BCC 68868	4.01	4.01	0.00	100:0	0.45
Lactococcus lactis BCC 68870	3.63	3.63	0.00	100:0	0.39
Lactococcus lactis BCC 68872	3.77	3.77	0.00	100:0	0.40
Lactococcus lactis TBRC 3367	3.98	3.98	0.00	100:0	0.41
Leuconostoc lactis BCC 62792	4.46	0.00	4.46	0:100	0.35
Levilactobacillus brevis TBRC 7984	2.10	1.77	0.33	84:16	0.15
Levilactobacillus brevis TBRC 7969	3.23	1.75	1.48	54:46	0.23
Levilactobacillus brevis TBRC 1539	0.94	0.36	0.57	39:61	0.09
Weizmannia coagulans TBRC 6952	1.82	1.82	0.00	100:0	0.30
Weizmannia coagulans TBRC 6965	1.16	1.16	0.00	100:0	0.21
Weizmannia coagulans TBRC 8004	1.48	1.48	0.00	100:0	0.30
Weizmannia coagulans TBRC 8005	1.03	1.03	0.00	100:0	0.21

3.4 Enhancement of stereospecific LA production by pH control

According to the fermentation profiles, we observed that cell growth of *La. lactis* BCC 68868 and *Le. lactis* BCC 62792 was limited at around OD_{600} 1.00 - 1.55 (Figure 2). These growth profiles corresponded to differences in pH decreases during fermentation, which pH of *La. lactis* BCC 68868 and *Le. lactis* BCC 62792 decreased from 5.5 to 4.5 (Figure 2A and 2B). To elucidate whether the acidic pH inhibited growth of *La. lactis* BCC 68868 and *Le. lactis* BCC 62792, which in turn limited LA production and further pH decrease, 1% $CaCO_3$ was added to the SCT medium to maintain pH during LA fermentation (Yang et al., 2015). After the addition of $CaCO_3$, the pH was maintained at 5.0-5.3 (Figure 3A and 3B). Upon addition of $CaCO_3$, the growth of *La. lactis* BCC 68868 and *Le. lactis* BCC 62792 increased to OD_{600} of 2.0 (Figure 2A and 2B) The increased cell growth greatly affected glucose consumption and LA production. After 24 h of cultivation, glucose was entirely consumed, and maximal yields of LA were achieved (Figure 2A and 2B). Under these conditions, *La. lactis* BCC 68868 almost utilizing glucose from non-detoxified SCT hydrolysate produced 19.34 $\pm$ 0.24 g/L L-LA, which is equivalent to a 3.8-fold improvement in the yield obtained. In addition to SCT, LA production achieved by LAB strains was established on various lignocellulosic biomass such as corncob, rice straw, and sugarcane bagasse. However, less of them validated on stereospecificity of LA products. This work is the first report on the converting of a bio-waste feedstock, SCT to the stereospecific L-LA or D-LA. *La. lactis* BCC 68868 produced L-LA as the primary product, suggesting its homofermentative pathway (Abedi and Hashemi, 2020). Compared to LAB capable of L-LA bioconversion from SB hydrolysate, the conversion yield of L-LA from *Le. lactis* BCC 68868 was 0.95 g L-LA/g sugar,

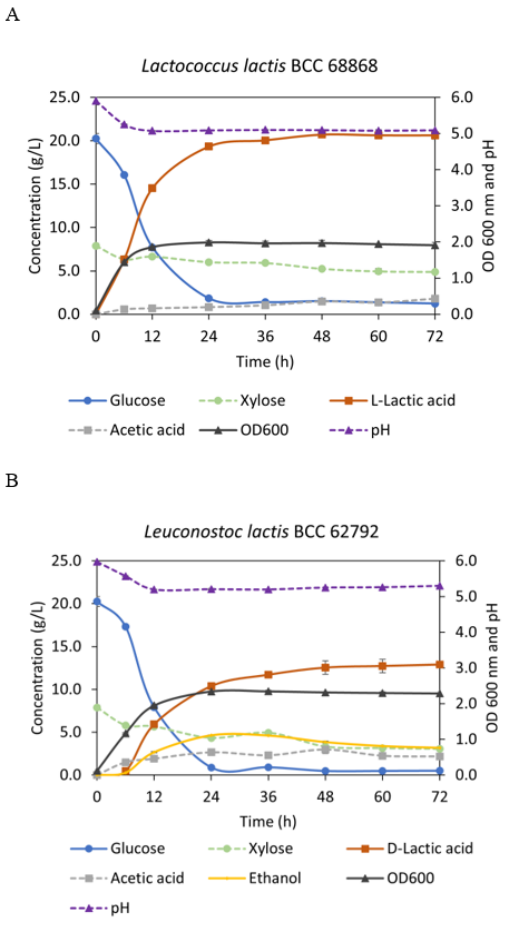

Figure 3. Fermentation profiles of *Lactococcus lactis* BCC 68868 (A) and *Leuconostoc lactis* BCC 62792 (B) grown on the SCT hydrolysate supplemented with nitrogen sources and $CaCO_3$.

Table 3. Screening of LAB strains potentially useful for SCT hydrolysate fermentation.

Strain Name	Substrates	Pretreatment methods	Fermentation processes	LA product	Titer (g/L)	Yield (g LA/g sugar)	References
Lactococcus lactis BCC 68868	Sugarcane trash	Liquid hot water	Batch (250 mL flask)	L-LA	19.3	0.95	This work
Lactobacillus sp. 17C5	Sugarcane bagasse	Acid hydrolysis	Batch (2 L fermentor)	L-LA	55.5	0.90	(Patel et al., 2004)
Lactobacillus coagulans DSM ID 14-300	Sugarcane bagasse	Acid hydrolysis	Batch (0.5 L fermentor)	L-LA	56.0	0.87	(Alves et al., 2019)
Lactobacillus sp. P38	Sugarcane bagasse	Acid hydrolysis	Fed-batch	L-LA	185.0	0.99	(Peng et al., 2014)
Lactobacillus casei ATCC 334	Sugarcane bagasse	Ionic liquid/Acid hydrolysis	SSF	L-LA	204.1	0.92	(Qureshi et al., 2023)
Leuconostoc lactis BCC 62792	Sugarcane trash	Liquid hot water	Batch (250 mL flask)	D-LA	10.4	0.45	This work
Lactobacillus delbrueckii NBRC 3534	Sugarcane bagasse	Steam explosion	Batch (250 mL flask)	D-LA	16.5	0.79	(Sasaki et al., 2012)
Pediococcus acidilactici XH11	Sugarcane bagasse	Acid hydrolysis	SSCF	D-LA	57.0	NR	(Qiu et al., 2023)

which is higher than the conversion yields from *Lactobacillus sp*. 17C5 and *B. coagulans* DSM ID 14-300, which are 0.90 and 0.87 g LA/g sugar, respectively (Patel et al., 2004; Alves de Oliveira et al., 2019) (Table 3). Nevertheless, the titer (g/L) of L-LA from *La. lactis* BCC 68868 was lower with the effect of the fermentation scale. Application of Fed-batch fermentation and simultaneous saccharification and fermentation (SSF) exhibited an increasing of titer of L-LA from *Lactobacillus sp*. P38 and *L. casei* ATCC 334 up to 185.0 and 204.1 g L-LA/g sugar (Peng et al., 2014; Qureshi et al., 2023) (Table 3).

For *Le. lactis* BCC 62792, the addition of $CaCO_3$ maintained the pH at 5.3 during fermentation. This led to an increase in glucose consumption from 12.9 g/L to 19.8 g/L, while a minor consumption of approximately 4.8 g/L of xylose, a previously unused C5-carbon source, was observed (Figure 3B). Therefore, the addition of $CaCO_3$ was not only to control pH but could enhance carbon utilization which led to an increase in D-LA yield from 6.50 $\pm$ 0.06 g/L to 10.39 $\pm$ 0.04 g/L. However, the conversion yield slightly decreased from 0.47 g LA/g sugar to 0.45 g LA/g sugar, possibly because most of the utilized carbon was converted to by-products such as acetic acid and ethanol (Figure 3B). These results indicated that *Le. lactis* BCC 62792 is heterofermentative LAB with a metabolic process that utilizes both glucose and xylose to LA (Oshiro et al., 2009), with glucose being the preferred carbon source. Assimilation of xylose was found after 6 hours, by which point 14.5% glucose had been consumed. These observations agree with a previous report where *Le. lactis* SHO-47 and *Le. lactis* SHO-54 produced D-LA from a mixture of sugar via NAD-specific 6-phosphogluconate dehydrogenase (Ohara et al., 2006; Ohara et al., 1998). In terms of D-LA production, *Le. lactis* BCC 62792 exhibited a relatively low yield of D-LA (0.47 g LA/g sugar) compared to *L. delbrueckii* NBRC 3534 which achieved a D-LA yield of 0.79 g LA/g sugar (Sasaki et al., 2012) (Table 3). It is important to note that the study utilizing *L. delbrueckii* NBRC 3534 only utilized 28% of sugarcane bagasse hydrolysate, indicating that inhibitory by-products present in the hydrolysate may have impacted sugar utilization. *P. acidilactici* XH11 utilized SB hydrolysate with the SSCF process to produce D-LA with a high titer of up to 57.0 g/L (Qiu et al., 2023) (Table 3). Increasing the starting glucose concentration in SCT hydrolysates or the application of a fed-batch fermentation process are considered promising approaches to further increase the concentration of LA.

4 Conclusions

This study is the first to demonstrate the direct use of SCT hydrolysates as a raw material for stereospecific LA fermentation with no prior detoxification step. LHW pretreatment and pH control enabled LAB strains *La. lactis* BCC 68868 and *Le. lactis* BCC 62792 to completely utilize glucose from non-detoxified SCT hydrolysates and successfully convert sugars to either optically pure L-LA or D-LA. Further optimization of the fermentation process to improve the product yield is warranted.

Author Contributions

KP, KB, NA, TP, BB, MR, KK & VC conceptualized and designed the study; KP, KB, TP, BB, MR performed research and analyzed data; and KP, KB, NA, BB, MR, KK & VC wrote the paper. All authors reviewed and edited the paper.

Conflict of Interest

No conflict of interest was reported by all authors.

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